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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 28 October 2003 with an application for Letters Patent number 529220 made by Garth James Smith COOPER; Yu WANG and Aimin XU.

I further certify that pursuant to a claim under Section 24(1) of the Patents Act 1953, a direction was given that the application proceed in the name of PROTEMIX CORPORATION LIMITED.

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PATENTS ACT, 1953

PROVISIONAL SPECIFICATION

"A Novel Peptide and its Uses thereof"

We, **GARTH JAMES SMITH COOPER**, a New Zealand citizen of 2 Crummer Road, Ponsonby, Auckland, New Zealand, **YU WANG**, a New Zealand citizen of 447A Dominion Road, Mt Eden, Auckland, New Zealand and **AIMIN XU**, a New Zealand citizen of 447A Dominion Road, Mt Eden, Auckland, New Zealand and 447A Dominion Road, Mt Eden, Auckland, New Zealand, do hereby declare this invention to be described in the following statement:

A NOVEL PEPTIDE & ITS USES THEREOF

FIELD OF THE INVENTION

The subject invention relates to a novel peptide and its uses thereof. More particularly to polypeptides and related molecules with proposed utility in the treatment of obesity and/or other related conditions associated with increased mass of adipocytes. The invention has, for example, application in the biomedical sciences.

BACKGROUND

Insulin resistance, is reported to be characterized by diminished sensitivity of insulin in its target tissues, is reportedly a fundamental aspect of the aetiology of type 2 diabetes and is often associated with other diseases such as, for example, hyperlipidaemia, atherosclerosis and hypertension (i.e., syndrome X). Although the molecular basis of insulin resistance is extremely complex and multifactorial, growing evidence suggests that altered function of adipocytes may play an important role in this process. A close correlation is reported to exist between changes in fat mass and insulin sensitivity. Insulin resistance and hyperinsulinaemia is reported to occur in obese as well as in lipodystrophic individuals. The pivotal role of adipocytes in the regulation of insulin sensitivity is further supported by two recent independent genetic studies on fat-free mice, which have severe insulin resistance and hyperglycemia. Furthermore, insulin-sensitizing drugs that target transcriptional regulation of adipocytes also link insulin sensitivity to adipocyte function.

Adipose tissue is reported to serve as an energy storage depot for triglycerides. It is also reported to be an active endocrine organ that can secrete a variety of biologically active molecules in response to extracellular signals. These adipocyte-secreted products have been reported to play critical roles in the regulation of systematic energy homeostasis, and their altered expression and/or secretion are reported to contribute to the causation of insulin resistance and its associated syndromes. One of the well-reported adipocyte-secreted products is leptin, which is reported to be a central regulator of adiposity and also affects glucose homeostasis. Some of the adipocyte-secreted molecules, such as TNFα, free fatty acid, and the recently characterized resistin, may have a direct role in causation of insulin resistance and its related syndromes. TNFα, which is reported to be overproduced by adipose tissue in insulin resistant states, may directly impede the insulin-signalling cascade. Furthermore, the increased expression and secretion of plasminogen activator inhibitor 1 (PAI-1) and angiotensinogen in the adipose tissue may play an important role in linking obesity to thrombotic vascular disease and hypertension.

The role of adipose tissue as an endocrine organ has been further reinforced by the recent discovery of adiponectin, a hormone exclusively secreted from adipocytes. It is reported that Messenger RNA (mRNA) expression and the secretion level of adiponectin are dramatically decreased in a variety of animal models with insulin resistance, as well as in obese humans and type

2 diabetic patients from different ethnic groups. Further, it is reported that replenishment of adiponectin decreased hyperglycemia, restored insulin resistance and caused sustained weight loss in mice without affecting food intake. This has been suggested in the literature that this protein is a potential insulin sensitizer.

Despite the identification of numerous secretory factors, a recently reported adipocyte-specific GLUT4 knockout study suggests that there are other yet-unidentified factors which may play important roles in modulating energy metabolism.

SUMMARY OF THE INVENTION

In one aspect, the invention provides an isolated polynucleotide that encodes, or is complementary to a sequence that encodes, an adipocyspin polypeptide. The invention encompasses a polynucleotide comprising a sequence encoding a polypeptide that has an anti-obesity activity, or a fragment thereof, and which is: (a) a polynucleotide having the sequence of

MKCLLISLALWLGTVGTRGTEPELSETQRRSLQVALEEFHKHPPVQLA
FQEIGVDRAEEVLFSAGTFVRLEFKLQQTNCPKKDWKKPECTIKPNGR
RRKCLACIKMDPKGKILGRIVHCPILKQGPQDPQELQCIKIAQAGEDP
HGYFLPGQFAFSRALRTK SEQ ID NO: 1;

and/or (b) a polynucleotide which hybridizes under stringent conditions to a polynucleotide of (a); and/or (c) a polynucleotide sequence which is degenerate as a result of the genetic code to the sequences defined in (a) or (b). In some embodiments the polynucleotide has at least 10, 15, 25, 50 or 100 contiguous bases identical or exactly complementary to SEQ ID NO:1. In various embodiments, the polynucleotide is the full-length sequence of SEQ ID NO:1 or encodes an adipocyspin polypeptide having the sequence of SEQ ID:2 or a fragment thereof.

The polynucleotide of the invention may be operably linked to a promoter or other sequence that enhances expression of the polynucleotide in a cell, such as an adipocyte or a 3T3 L1 cell. In another embodiment, the invention provides recombinant vector (*e.g.*, an expression vector) expressing an adipocyspin polypeptide or fragment. The invention further provides a cell (*e.g.*, a bacterial, eukaryotic; mammalian, or human cell) containing a recombinant adipocyspin polynucleotide of the invention; and provides a method for producing an adipocyspin protein, peptide, or fusion protein by culturing a cell containing the recombinant adipocyspin polynucleotide under conditions in which the polypeptide is expressed.

In another embodiment, the invention provides an isolated, substantially pure, or recombinant adipocyspin polypeptide, or immunogenic fragment thereof. In one embodiment the polypeptide has the amino acid sequence identical to SEQ ID NO:1. In another embodiment, the polypeptide with an amino acid sequence that differs from SEQ ID NO:1 by conservative

mutations, which is at least 60%, 80%, or 90% identical to SEQ ID NO:1, and/or that is immunologically cross-reactive with the full-length polypeptide encoded by SEQ ID NO:1. In one embodiment, the polypeptide of the invention is a fusion protein. In some embodiments, the polypeptide of the invention has an activity of a naturally occurring human adipocyspin, such as inhibiting the formation of adipocytes from preadipocytes, or decreasing body adiposity or adipose tissue mass.

In another embodiment, the invention provides an antibody, or antibody fragment (*e.g.*, Fab fragment or single chain antibody) or binding fragment (*e.g.*, produced by phage display) that specifically binds to the adipocyspin polypeptide of the invention. The antibody may be monoclonal and may bind with an affinity of at least about 10^8 M^{-1}. The invention also provides an isolated cell or a hybridoma capable of secreting the antibody. The antibody may be human or humanized.

In another aspect the invention provides a method of detecting an adipocyspin gene product in a sample by (a) contacting the sample with a probe that specifically binds the gene product, wherein the probe and the gene product form a complex, and detecting the formation of the complex; or (b) specifically amplifying the gene product in the biological sample, wherein said gene product is a polynucleotide, and detecting the amplification product; wherein the formation of the complex or presence of the amplification product is correlated with the presence of the adipocyspin gene product in the biological sample. In one embodiment the gene product is a polypeptide and probe is an antibody. In a different embodiment, the gene product is an RNA and the probe is a polynucleotide.

In one aspect, the invention provides a method for identifying a modulator of **adipocyspin** activity by (a) contacting a polypeptide encoding **adipocyspin** and the adipocyspin receptor in the presence of a test compound. and (b) comparing the level of binding of the adipocyspin receptor and the polypeptide in (a) with the level of binding in the absence of the test compound, wherein a decrease in binding indicates that the test compound is an inhibitor of binding and an increase in binding indicates that the test compound is an enhancer of binding. In an embodiment, the **adipocyspin** polypeptide is expressed by a cell.

In a related aspect, the invention provides a method of identifying a modulator of adipocyspin activity by contacting a cell expressing a recombinant adipocyspin polypeptide and a test compound and assaying for a biological effect that occurs in the presence but not absence of the test compound, wherein a test compound that induces a biological effect is identified as a modulator of adipocyspin activity. In one embodiment, the biological effect assayed for is the rate of conversion of preadipocytes to adipocytes.

In another related aspect, the invention provides a process for making a pharmaceutical composition by formulating a modulator of **adipocyspin** activity (*e.g.* binding) for pharmaceutical

use.

Another aspect the invention also provides a method for identifying compounds which will be useful for the treatment of **adipocyspin**-mediated diseases and conditions, by determining whether the compound interacts with the **adipocyspin**.

In another aspect, the invention provides a method of treating an adipocyspin-mediated condition in a mammal by reducing or increasing the activity or expression of adipocyspin in a cell or tissue in the mammal or administering a modulator of adipocyspin function to the mammal. In various embodiments, the adipocyspin-mediated condition or disease is obesity or conditions associated with increased adipose tissue mass.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows 2-DE separation of a low molecular weight adipocyte-secreted protein induced during adipose conversion where after 8 days of induction of differentiation subconfluent 3T3-L1 preadipocytes or adipocytes were rinsed three times with PBS, and then incubated with serum free DMEM for 4 hr. The medium was collected, concentrated, and 50 μg of proteins from each sample were separated by 2-DE and visualized with silver staining and the protein preferentially secreted in the adipocytes is denoted as the arrow.

Figure 2 shows, Microcharacterization of the novel adipocyte-secreted product by reversed phase HPLC and amino acid sequencing. The spots corresponding to the adipocyte-specific protein as from Figure 1 were excised from multiple Coommassie Brilliant Blue stained gels and subjected to trypsin digestion. The tryptic peptide mixture was separated by RP HPLC to fractionate the peptides and the table shows the amino acid sequences for the indicated RP HPLC fractions.

Figure 3 shows the, Sequence analysis of adipocyspin. (A) the amino acid sequence of mouse adipocyspin, (B) is the schematic diagram of mouse adipocyspin and (C) is the alignment of cystatin-like domains of mouse adipocyspin and mouse cystatin C.

Figure 4 shows that Adipocyspin is secreted from transfected COS 7 cells. COS 7 cells were transiently transfected with a plasmid which encodes COOH terminal FLAG-tagged full-length adipocyspin which are grown in DMEM for 48 hours. The cell culture medium is then collected, and concentrated using Vivian concentrator (with MWCO of 5000 kDa). 20 μg of proteins from either cell pellet or medium are separated by SDS-PAGE and probed with either anti-FLAG monoclonal antibody or anti- β tubulin monoclonal antibody. It is to be noted that, in cell culture medium, adipocyspin can be readily detected in cell medium while β tubulin can not be detected.

Figure 5 shows the Differentiation-dependent expression of adipocyspin mRNA where

the Total RNA was purified from NIH 3T3 cells or from 3T3-L1 cells at the indicated time points following hormonal differentiation. 10 μg of total RNA from each sample was subjected to Northern blot analysis, using ^{32}P labelled adipocyspin DNA. As a control for RNA loading the 18 S RNA hybridization signal is shown alongside the results of adipocyspin.

Figure 6 shows the dysregulation of the adipocyspin gene expression in obese mouse (ob/ob) where 10 μg of total RNA from lean (ob/+) or obese (ob/ob) fat pads were analysed by Northern blot analysis. The same membrane was probed with ^{32}P labelled cDNAs corresponding to adipocyspin and adiponectin, as indicated. As in figure 5, as a control for RNA loading the 18 S RNA hybridization signal is shown.

Figure 7 shows that Adipocyspin inhibits the differentiation of 3T3 L1 preadipocytes. FLAG tagged adipocyspin was purified from transiently transfected COS 7 cells as in Fig. 4. 3T3 L1 cells were induced for differentiation in the absence of 20 μg/m FLAG-tagged adipocyspin the results are shown in A or in the presence of 20 μg/ml FLAG-tagged adipocyspin the results are in B, where each slide is taken at day 6 after differentiation, the cells were stained with oil Red O and visualized by light microscopy, and

Figure 8 shows that adipocyspin inhibits the expression of adipocyte-specific gene products in adipocytes where 3T3 L1 cells were differentiated in the absence (lane 1) or presence (lane 2) of 20 μg/ml FLAG tagged adipocyspin as in Figure 7 the slides are taken 6 days after differentiation, where 10 μg of total RNA from these cells was analysed by Northern blotting as in figure 5 and probed with ^{32}P-labelled cDNAs corresponding to PPARγ or GLUT4.

DETAILED DESCRIPTION OF THE INVENTION

Adipocyte-secreted products have recently been reported to play a critical role in regulating energy metabolism as well as physiological processes, such as modulating vascular tone and the acute-phase response. We have shown the identification and characterisation of a novel adipocyte-secreted factor, which we have named 'adipocyspin'.

We have identified this protein by the use of a two-dimensional gel electrophoresis to show that a protein (with apparent molecular mass of 17 kDa and pI value of 9.4) is expressed and secreted preferentially from 3T3 L1 adipocytes but not from corresponding preadipocytes. Amino acid sequence analysis and cDNA cloning revealed this protein and to contain a putative secretory signal peptide, followed by a domain which shares sequence homology with families of cysteine protease inhibitors. The adipocyspin mRNA was virtually undetectable in 3T3 L1 preadipocytes and markedly increased following hormone induced adipose conversion. Its expression in adipose tissue is shown to significantly increase in obese states. The conversion of 3T3-L1 cells to

adipocytes was dramatically inhibited following treatment with FLAG tagged adipocyspin purified from transiently transfected COS 7 cells. The regulated expression pattern and the inhibitory effect on adipocyte conversion implicates that adipocyspin might function as a feedback regulator of adipogenesis.

We have shown that adipocyspin down-regulates the conversion of preadipocytes into adipocytes. It is expected to have utility in states associated with increased rates of preadipocytes to adipocyte conversion, such as those associated with adipocyte hyperplasia.

II. ADIPOCYSPIN POLYNUCLEOTIDES

In one aspect, the invention provides a polynucleotide having a sequence or subsequence of a mammalian (*e.g.*, rat or human) adipocyspin gene or RNA. The polynucleotides of the invention *(e.g.,* RNA, DNA, PNA or chimeras), may be single-stranded, double stranded, or a mixed hybrid. In one embodiment, the polynucleotide has a sequence of SEQ. ID NO: 1 (Figure 1) or subsequences thereof (*e.g.*, comprising at least 15, at least 25, at least 50, at least 100, at least 200, or at least 500 bases of the polynucleotides and variants of the invention). The invention also provides polynucleotides with substantial sequence identity to the adipocyspin polynucleotides disclosed herein. Thus, the invention provides naturally occurring alleles of mammalian (*e.g.*, human) adipocyspin genes such as human allelic variants of the adipocyspin polynucleotides of SEQ ID NO:1.

As described *infra,* in some embodiments the polynucleotide of the invention encodes a polypeptide with substantial sequence similarity to SEQ. ID NO:1 (Figure 1) or encodes a fragment of such a polypeptide (*e.g.*, a fusion protein). Also contemplated are polynucleotides that, due to the degeneracy of the genetic code, are not substantially similar to SEQ. ID NO:1, but encode the polypeptide of SEQ. ID NO:1 or a fragment thereof. In other embodiments, the invention provides adipocyspin polynucleotides that do not necessarily encode adipocyspin polypeptide but which are useful as *e.g.,* probes, primers, antisense, triplex, or ribozyme reagents, and the like.

The invention also includes expression vectors, cell lines, and transgenic organisms comprising the adipocyspin polynucleotides. In some embodiments, the vectors, cells, and organisms of the invention are capable of expressing the encoded adipocyspin polypeptides.

The adipocyspin polynucleotides of the invention can be produced by recombinant means. See, *e.g.*, Sambrook *et al.*, Berger and Kimmel, (1987) Methods In Enzymology, Vol. 152: Guide To Molecular Cloning Techniques, San Diego: Academic Press, Inc.; Ausubel *et al.*, Current Protocols In Molecular Biology, Greene Publishing and Wiley-Interscience, New York (2001). Alternatively, adipocyspin polynucleotides or fragments can be chemically synthesized using routine methods well known in the art *(see, e.g.,* Narang *et al.*, 1979, *Meth. Enzymol.* 68:90; Brown

et al. 1979, *Meth. Enzymol.* 68:109; Beaucage et al., 1981, *Tetra. Lett.*, 22:1859). In some embodiments, the adipocyspin polynucleotides of the invention contain non-naturally occurring bases, *e.g.*, deoxyinosine (see, Batzer et al., 1991, *Nucleic Acid Res.* 19:5081; Ohtsuka et al., 1985,.J. *Biol. Chem.* 260:2605- 2608; Rossolini et al., 1994, *Mol. Cell. Probes* 8:91-98) or modified backbone residues or linkages.

A. Polynucleotides Encoding adipocyspin

In one aspect, the invention provides polynucleotides encoding adipocyspin polypeptides such as an adipocyspin polypeptide having the sequence of SEQ ID NO:1, a fragment thereof, a variant thereof *(e.g.,* a conservative or allelic variant), or an adipocyspin fusion polypeptide. In one embodiment, the polynucleotide of the invention comprises the sequence of SEQ ID NO:1 or a fragment thereof. In another embodiment, the polynucleotide encodes a naturally occurring adipocyspin polypeptide or fragment, but has a sequence that differs from SEQ. ID NO:1 *(e.g.,* as a result of the degeneracy of the genetic code). In some embodiments of the invention, the polynucleotide is other than the expressed sequence tags H67224, AI1131555, AA215577, AW190975 or AI769466 or the polynucleotide encoding bovine PPR1 (Matsuoka et al,., 1993, *Biochem Biophys Res Comm* 194:540-11).

The polynucleotides of invention are useful for expression of adipocyspin polynucleotides (*e.g.*, sense or antisense RNAs) and polypeptides. Methods for recombinant expression of polynucleotides and polypeptides are well known in the art. Typically, the adipocyspin polynucleotides of the invention are used in expression vectors for the preparation of adipocyspin polypeptides and polynucleotides. Expression vectors typically include transcriptional and/or translational control signals *(e.g.,* transcriptional regulatory element, promoter, ribosome-binding site, and ATG initiation codon). In addition, the efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use. For example, the SV40 enhancer or CMV enhancer can be used to increase expression in mammalian host cells.

Thus, in one embodiment, DNA encoding an adipocyspin polypeptide of the present invention is inserted into DNA constructs capable of introduction into and expression in an *in vitro* host cell, such as a bacterial *(e.g., E. coli, Bacillus subtilus),* yeast *(e.g., Saccharomyces),* insect *(e.g., Spodoprera frugiperda),* or mammalian cell culture systems. Examples of mammalian cell culture systems useful for expression and production of the polypeptides of the present invention include human embryonic kidney line (293; Graham et al., 1977, .I: *Gen. Virol.* 36:59); CHO (ATCC CCL 61 and CRL 9618); human cervical carcinoma cells (He La, ATCC CCL 2); and others known in the art. Useful human and nonhuman cell lines are widely available, *e.g.*, from the American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108 (see

http://www.atcc.org). The use of mammalian tissue cell culture to express polypeptides is discussed generally in Winnacker, FROM GENES TO CLONES (VCH Publishers, N.Y., N.Y., 1987) and Ausubel, *supra.*

In some embodiments, promoters from mammalian genes or from mammalian viruses are used, *e.g.*, for expression in mammalian cell lines. Suitable promoters can be constitutive, cell type-specific, stage-specific, and/or modulatable or regulatable *(e.g.,* by hormones such as glucocorticoids). Useful promoters include, but are not limited to, the metallothionein promoter, the constitutive adenovirus major late promoter, the dexamethasone-inducible MMTV promoter, the SV 40 promoter, and promoter-enhancer combinations known in the art.

Adipocyspin and polypeptides or fragments can also be expressed in transgenic animals (mouse, sheep, cow, etc.) and plants (tobacco, arabidopsis, etc.) using appropriate expression vectors which integrate into the host cell chromosome.

B. Polynucleotide or Oligonucleotide Probes and Primers

In one embodiment, the invention provides oligonucleotide or polynucleotide probes and/or primers for detecting or amplifying adipocyspin polynucleotides. In various embodiments, the polynucleotides (*e.g.*, probes and primers) comprise at least 10 contiguous bases identical or exactly complementary to SEQ ID NO:1, usually at least 12 bases, typically at least 15 bases, generally at least 18 bases and often at least *25,* at least 50, or at least 100 bases. When the adipocyspin polynucleotides of the invention are used as probes or primers they are generally less that about 3000 bases in length; typically they contain between about 12 and about 500 contiguous nucleotides identical or exactly complementary to SEQ. ID NO:1, more often between about 12 and about 50 contiguous nucleotides, even more often between about 15 and about 25 contiguous nucleotides.

In some embodiments, the probes and primers are modified, *e.g.*, by adding restriction sites to the probes or primers. In other embodiments, primers or probes of the invention comprise additional sequences, such as linkers. In still some other embodiments, primers or probes of the invention are modified with detectable labels. For example, the primers and probes are chemically modified, *e.g.,* derivatized, incorporating modified nucleotide bases, or containing a ligand capable of being bound by an anti-ligand *(e.g.,* biotin).

The adipocyspin probes and primers of the invention can be used for a number of purposes, e.g., for detecting or amplifying an adipocyspin polynucleotide in a biological sample, as discussed in more detail infra. For example, provided with the guidance herein, one of skill will be able to select primer pairs that specifically amplify all or a portion of the adipocyspin gene, mRNA, or cDNA in a sample. In a preferred embodiment, the primer pairs and amplification conditions are chosen to not amplify other messenger RNAs present in the sample, e.g., due to 3' mismatch

between the **adipocyspin** primers and other gene sequences.

C. **Adipocyspin** Inhibitory Polynucleotides

The invention provides inhibitory polynucleotides such as antisense, triplex, and ribozyme reagents that target or hybridize to adipocyspin polynucleotides.

1. Antisense Polynucleotides

In one aspect, the present invention provides antisense oligonucleotides and polynucleotides that can be used to inhibit expression of the adipocyspin gene. Some therapeutic methods of the invention, described in additional detail *infra,* involve the administration of an oligonucleotide that functions to inhibit or stimulate adipocyspin activity under *in vivo* physiological conditions, and is relatively stable under those conditions for a period of time sufficient for a therapeutic effect. Polynucleotides can be modified to impart such stability and to facilitate targeting delivery of the oligonucleotide to the desired tissue, organ, or cell.

The antisense polynucleotides of the invention comprise an antisense sequence of at least about 10 bases~ typically at least 12 or 14, and up to about 3000 contiguous nucleotides that specifically hybridize to a sequence from mRNA encoding adipocyspin or mRNA transcribed from the adipocyspin gene. More often, the antisense polynucleotide of the invention is from about 12 to about 50 nucleotides in length or from about 15 to about 25 nucleotides in length. In general, the antisense polynucleotide should be long enough to form a stable duplex but short enough, depending on the mode of delivery, to administer *in vivo;* if desired. The minimum length of a polynucleotide required for specific hybridization to a target sequence depends on several factors, such as G/C content, positioning of mismatched bases (if any), degree of uniqueness of the sequence as compared to the population of target polynucleotides, and chemical nature of the polynucleotide (*e.g.*, methylphosphonate backbone, peptide nucleic acid, phosphorothioate); among other factors.

Generally, to assure specific hybridization, the antisense sequence is substantially complementary to the target adipocyspin mRNA sequence. In certain embodiments, the antisense sequence is exactly complementary to the target sequence. The antisense polynucleotides may also include, however; nucleotide substitutions, additions, deletions, transitions, transpositions, or modifications, or other nucleic acid sequences or non-nucleic acid moieties so long as specific binding to the relevant target sequence corresponding to adipocyspin RNA or its gene is retained as a functional property of the polynucleotide.

In one embodiment; the antisense sequence is complementary to relatively accessible sequences of the adipocyspin mRNA (*e.g.*; relatively devoid of secondary structure). This can be

determined by analyzing predicted RNA secondary structures using; for example, the MFOLD program (Genetics Computer Group, Madison WI) and testing *in vitro* or *in vivo* as is known in the art. Another useful method for identifying effective antisense compositions uses combinatorial arrays of oligonucleotides (see, *e.g.*, Milner et al., 1997, *Nature Biotechnology* 15:537).

The invention also provides an antisense polynucleotide that has sequences in addition to the antisense sequence (i.e., in addition to anti-adipocyspin-sense sequence). In this case, the antisense sequence is contained within a polynucleotide of longer sequence. In another embodiment, the sequence of the polynucleotide consists essentially of, or is, the antisense sequence.

The antisense nucleic acids (DNA, RNA, modified, analogues, and the like) can be made using any suitable method for producing a nucleic acid, such as the chemical synthesis and recombinant methods disclosed herein. In one embodiment, for example, antisense RNA molecules of the invention may be prepared by *de novo* chemical synthesis or by cloning. For example, an antisense RNA that hybridizes to adipocyspin mRNA can be made by inserting (ligating) an adipocyspin DNA sequence (*e.g.*, SEQ. ID NO:1, or fragment thereof) in reverse orientation operably linked to a promoter in a vector (*e.g.*. plasmid). Provided that the promoter and, preferably termination and polyadenylation signals, are properly positioned, the strand of the inserted sequence corresponding to the noncoding strand will be transcribed and act as an antisense oligonucleotide of the invention. The antisense oligonucleotides of the invention can be used to inhibit adipocyspin activity in cell-free extracts, cells, and animals, including mammals and humans.

For general methods relating to antisense polynucleotides, see ANTISENSE RNA AND DNA, (1988), D.A. Melton, Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and Dagle et al., 1991, *Nucleic Acids Research,* 19:1805. For a review of antisense therapy, see, *e.g.*, Uhlmann et aI., *Chem. Reviews,* 90:543-584 (1990).

2. Triplex Oligo- and Polynucleotides

The present invention provides oligo- and polynucleotides (*e.g.*, DNA, RNA, PNA 10 or the like) that bind to double-stranded or duplex adipocyspin nucleic acids (*e.g.*, in a folded region of the adipocyspin RNA or in the adipocyspin gene), forming a triple helix-containing, or "triplex" nucleic acid. Triple helix formation results in inhibition of adipocyspin expression by, for example, preventing transcription of the adipocyspin gene thus reducing or eliminating adipocyspin activity in a cell. Without intending to be bound by any particular mechanism, it is believed that triple helix pairing compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules to occur.

Triplex oligo- and polynucleotides of the invention are constructed using the base-pairing rules of triple helix formation (see, *e.g.*, Cheng et al., 1988. J: *Biol. Chem.* 263: 15110; FeIrin and Camerini-Otero. 1991, *Science* 354:1494; Ramdas et al., 1989, J: *Biol. Chem* 264:17395; Strobel et

al., 1991, *Science* 254:1639; and Rigas et al., 1986, *Proc. Natl. A cad. Sci. US.A.* 83: 9591; each of which is incorporated herein by reference) and the adipocyspin mRNA and/or gene sequence. Typically, the triplex-forming oligonucleotides of the invention comprise a specific sequence of from about 10 to at least about 25 nucleotides or longer "complementary' to a specific sequence in the adipocyspin RNA or gene (i.e., large enough to form a stable triple helix, but small enough, depending on the mode of delivery, to administer *in vivo,* if desired). In this context, "complementary" means able to form a stable triple helix. In one embodiment, oligonucleotides are designed to bind specifically to the regulatory regions of the adipocyspin gene (*e.g.*, the adipocyspin 5'-flanking sequence, promoters, and enhancers) or to the transcription initiation site, (*e.g.*, between -10 and +10 from the transcription initiation site). For a review of recent therapeutic advances using triplex DNA, see Gee et al.. *in* Huber and Carr, 1994, MOLECULAR. AND IMMUNOLOGIC APPROACHES, Futura Publishing Co, Mt Kisco NY and Rininsland et al., 1997, *Proc. Natl. Acad. Sci. USA* 94:5854, which are both incorporated herein by reference.

3. Ribozymes

The present invention also provides ribozymes useful for inhibition of adipocyspin activity. The ribozymes of the invention bind and specifically cleave and inactivate adipocyspin mRNA. Useful ribozymes can comprise 5'- and 3'-terminal sequences complementary to the adipocyspin mRNA and can be engineered by one of skill on the basis of the adipocyspin mRNA sequence disclosed herein (see PCT publication WO 93/23572, *supra).* Ribozymes of the invention include those having characteristics of group I intron ribozymes (Cech, 1995, *Biotechnology* 13:323) and others of hammerhead ribozymes (Edgington, 1992, *Biotechnology* 10:256).

Ribozymes of the invention include those having cleavage sites such as GUA~ GUU and GUC. Other optimum cleavage sites for ribozyme-mediated inhibition of adipocyspin activity in accordance with the present invention include those described in PCT publications WO 94/02595 and WO 93/23569, both incorporated herein by reference. Short RNA oligonucleotides between 15 and 20 ribonucleotides in length corresponding to the region of the target adipocyspin gene containing the cleavage site can be evaluated for secondary structural features that may render the oligonucleotide more desirable. The suitability of cleavage sites may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays, or by testing for *in vitro* ribozyme activity in accordance with standard procedures known in the art.

As described by Hu et al., PCT publication WO 94/03596, incorporated herein by reference, antisense and ribozyme functions can be combined in a single oligonucleotide. Moreover, ribozymes can comprise one or more modified nucleotides or modified linkages between

nucleotides, as described above in conjunction with the description of illustrative antisense oligonucleotides of the invention.

In one embodiment, the ribozymes of the invention are generated *in vitro* and introduced into a cell or patient. In another embodiment, gene therapy methods are used for expression of ribozymes in a target cell *ex vivo* or *in vivo*.

4. Administration of Oligonucleotides

Typically, the therapeutic methods of the invention involve the administration of an oligonucleotide that functions to inhibit or stimulate adipocyspin activity under *in vivo* physiological conditions, and is relatively stable under those conditions for a period of time sufficient for a therapeutic effect. As noted above, modified nucleic acids may be useful in imparting such stability, as well as for targeting delivery of the oligonucleotide to the desired tissue, organ, or cell.

Oligo- and poly-nucleotides can be delivered directly as a drug in a suitable pharmaceutical formulation or indirectly by means of introducing a nucleic acid into a cell, including liposomes, immunoliposomes, ballistics, direct uptake into cells, and the like as described herein. For treatment of disease, the oligonucleotides of the invention will be administered to a patient in a therapeutically effective amount. A therapeutically effective amount is an amount sufficient to ameliorate the symptoms of the disease or modulate adipocyspin activity in the target cell. Methods useful for delivery of oligonucleotides for therapeutic purposes are described in U.S. Patent 5,272,065, incorporated herein by reference. Other details of administration of pharmaceutically active compounds are provided below. In another embodiment, oligo- and poly-nucleotides can be delivered using gene therapy and recombinant DNA expression plasmids of the invention.

D. Gene Therapy

Gene therapy refers to the introduction of an otherwise exogenous polynucleotide which produces a medically useful phenotypic effect upon the (typically) mammalian cell(s) into which it is transferred. In one aspect, the present invention provides gene therapy methods and compositions for treatment of adipocyspin-associated conditions. In illustrative embodiments,
gene therapy involves introducing into a cell a vector that expresses an adipocyspin gene product (such as an adipocyspin protein substantially similar to the adipocyspin polypeptide having a sequence of SEQ. ID NO:1, *e.g.*, to increase adipocyspin activity, or an inhibitory adipocyspin polypeptide to reduce activity), expresses a nucleic acid having an adipocyspin gene or mRNA sequence (such as an antisense RNA, *e.g.*, to reduce adipocyspin activity), expresses a polypeptide or polynucleotide that otherwise affects expression of adipocyspin gene products (*e.g.*, a ribozyme

directed to adipocyspin mRNA to reduce adipocyspin activity), or replaces or disrupts an endogenous adipocyspin sequence (*e.g.*, gene replacement and gene knockout, respectively). Numerous other embodiments will be evident to one of skill upon review of the disclosure herein.

Vectors useful in adipocyspin gene therapy can be viral or nonviral, and include those described *supra* in relation to the adipocyspin expression systems of the invention. It will be understood by those of skill in the art that gene therapy vectors may comprise promoters and other regulatory or processing sequences, such as are described in this disclosure. Usually the vector will comprise a promoter and, optionally, an enhancer (separate from any contained within the promoter sequences) that serve to drive transcription of an oligoribonucleotide, as well as other regulatory elements that provide for episomal maintenance or chromosomal integration and for high-level transcription, if desired. A plasmid useful for gene therapy can comprise other functional elements, such as selectable markers, identification regions, and other sequences. The additional sequences can have roles in conferring stability both outside and within a cell, targeting delivery of adipocyspin nucleotide sequences (sense or antisense) to a specified organ, tissue, or cell population, mediating entry into a cell, mediating entry into the nucleus of a cell and/or mediating integration within nuclear DNA. For example, aptamer-like DNA structures, or other protein binding moiety sites can be used to mediate binding of a vector to cell surface receptors or to serum proteins that bind to a receptor thereby increasing the efficiency of DNA transfer into the cell. Other DNA sites and structures can directly or indirectly bind to receptors in the nuclear membrane or to other proteins that go into the nucleus, thereby facilitating nuclear uptake of a vector. Other DNA sequences can directly or indirectly affect the efficiency of integration.

Suitable gene therapy vectors may, or may not, have an origin of replication. For example, it is useful to include an origin of replication in a vector for propagation of the vector prior to administration to a patient. However, the origin of replication can often be removed before administration if the vector is designed to integrate into the host chromosomal DNA or bind to host mRNA or DNA.

As noted, the present invention also provides methods and reagents for gene replacement therapy (i.e., replacement by homologous recombination of an endogenous adipocyspin gene with a recombinant gene). Vectors specifically designed for integration by homologous recombination may be used. Important factors for optimizing homologous recombination include the degree of sequence identity and length of homology to chromosomal sequences. The specific sequence mediating homologous recombination is also important because integration occurs much more easily in transcriptionally active DNA. Methods and materials for constructing homologous targeting constructs are described by *e.g.*, Mansour et al., 1988, *Nature* 336: 348; Bradley et al., 1992, *Bio/Technology* 10: 534. See also, U.S. Patent Nos. 5,627,059; 5,487,992; 5,631,153; and

5,464,764. In one embodiment, gene replacement therapy involves altering or replacing all or a portion of the regulatory sequences controlling expression of the gene that is to be regulated. For example, the adipocyspin promoter sequences (as showing in Figure 5) may be disrupted to decrease adipocyspin expression or to abolish a transcriptional control site or an exogenous promoter (*e.g.*, to increase adipocyspin expression) substituted.

The invention also provides methods and reagents for adipocyspin "gene knockout" (i.e., deletion or disruption by homologous recombination of an endogenous adipocyspin gene using a recombinantly produced vector). In gene knockout, the targeted sequences can be regulatory sequences (*e.g.*, the adipocyspin promoter), or RNA or protein coding sequences. The use of homologous recombination to alter expression of endogenous genes is described in detail in U.S. Patent No. 5,272,071, WO 91/09955, WO 93/09222, WO 96/29411, WO 95/31560, and WO 91/12650. See also, Moynahan et al.; 1996, *Hum. Mol. Genet.* 5:875.

Gene therapy vectors may be introduced into cells or tissues *in vivo, in vitro* or *ex vivo*. For *ex vivo* therapy, vectors may be introduced into cells, *e.g.*, stem cells, taken from the patient and clonally propagated for autologous transplant back into the same patient (see, *e.g.*, U.S. Patent Nos. 5,399,493 and 5,437,994).

E. Transgenic Organisms

The invention also provides transgenic non-human multicellular organisms (*e.g.*, plants and non-human animals) or unicellular organisms (*e.g.*, yeast) comprising an exogenous adipocyspin gene sequence, which may be a coding sequence or a regulatory (*e.g.*, promoter) sequence. Examples of multicellular organisms include plants, insects, and nonhuman animals such as mice, rats, rabbits, monkeys, apes, pigs, and other nonhuman mammals. An example of a unicellular organism is a yeast. In one embodiment, the organism expresses an exogenous adipocyspin polypeptide, having a sequence of a human adipocyspin protein. The invention also provides unicellular and multicellular organisms (or cells therefrom) in which a gene encoding adipocyspin is mutated or deleted (i.e., in a coding or regulatory region) such that native adipocyspin is not expressed, or is expressed at reduced levels or with different activities when compared to wild-type cells or organisms. Such cells and organisms are often referred to as "gene knock-out" cells or organisms.

The invention further provides cells and organisms in which an endogenous adipocyspin gene is either present or optionally mutated or deleted and an exogenous adipocyspin gene or variant (*e.g.*, human adipocyspin) is introduced and expressed. Cells and organisms of this type will be useful, for example, as model systems for identifying modulators of adipocyspin activity or expression; determining the effects of mutations in the adipocyspin gene.

Methods for alteration or disruption of specific genes (*e.g.*, endogenous adipocyspin genes) are well ,known to those of skill, see, *e.g.*, Baudin et al., 1993. *Nucl. Acids Res.* 21:3329; Wach *et al.*, 1994, *Yeast* 10:1793; Rothstein, 1991, *Methods Enzymol.194:281;* Anderson, 1995, *Methods Cell Biol.* 48:31; Pettitt et *al..1996, Development* 122:4149-4157; Ramirez-Solis et al., 1993, *Methods Enzymol.* 225:855; and Thomas et al., 1987, *Cell* 51:503. Typically, such methods involve altering or replacing all or a portion of the regulatory sequences controlling expression of the particular gene to be regulated. The regulatory sequences, *e.g.* the native promoter can be altered. One conventional technique for targeted mutation of genes involves placing a genomic DNA fragment containing the gene of interest into a vector, followed by cloning of the two genomic arms associated with the targeted gene around a selectable neomycin-resistance cassette in a vector containing thymidine kinase. This "knock-out" construct is then transfected into the appropriate host cell, *i.e.*, a mouse embryonic stem (ES) cell, which is subsequently subjected to positive selection (using G418, for example, to select for neomycin-resistance) and negative selection (using, for example, FIAU to exclude cells lacking thymidine kinase), allowing the selection of cells which have undergone homologous recombination with the knockout vector. This approach leads to inactivation of the gene of interest. See, for example, U.S. patents 5,464,764; 5,631,153; 5,487,992; and, 5,627,059.

The "Knocking out" expression of an endogenous gene can also be accomplished by the use of homologous recombination to introduce a heterologous nucleic acid into the regulatory sequences (*e.g.*, promoter) of the gene of interest. To prevent expression of the functional enzyme or product, simple mutations that either alter the reading frame or disrupt the promoter can be suitable. To up-regulate expression, a native promoter can be substituted with a heterologous promoter that induces higher levels of transcription. Also, "gene trap insertion" can be used to disrupt a host gene, and mouse ES cells can be used to produce knockout transgenic animals, as described for example, in Holzschu (1997) *Transgenic Res* 6: 97-106. Other methods are known in the art.

Altering the expression of endogenous genes by homologous recombination can also be accomplished by using nucleic acid sequences comprising the structural gene in question. Upstream sequences are utilized for targeting heterologous recombination constructs. Utilizing adipocyspin structural gene sequence information, such as SEQUENCE ID NO:1, one of skill in the art can create homologous recombination constructs with only routine experimentation.

Homologous recombination to alter expression of endogenous genes is described in U.S. Patent 5,272,071, and WO 91/09955, WO 93/09222, WO 96/29411, WO *95/31560,* and WO 91/12650. Homologous recombination in animals has been described by Moynahan (1996) *Hum. Mol. Genet.* 5:875, and in plants by Offringa (1990) *EMBOJ.* 9:3077.

III. ADIPOCYSPIN POLYPEPTIDES

The present invention provides isolated, substantially pure, or recombinant adipocyspin polypeptides and immunogenic fragments of mammalian adipocyspin polypeptides. In one embodiment, the adipocyspin polypeptide or fragment has an amino acid sequence identical to, or substantially identical to, the sequence set forth in SEG ID NO:1 or a subsequence thereof.

A. Adipocyspin Polypeptides and Variants

The invention provides substantially pure, isolated, or recombinant adipocyspin polypeptides. In some embodiments, the adipocyspin polypeptide has an amino acid sequence identical or substantially identical to the amino acid sequence shown in SEQ ID NO:1. In other embodiments, the adipocyspin polypeptides are variants and mutants characterized by conservative substitutions of amino acid residues of SEQ ID NO:1.

The polypeptide of the invention may be full-length (*e.g.*, containing about 150 amino acids for the species shown in Fig. 3 or may encode a fragment of the full-length protein (*e.g.*, comprising at least 20, at least 40, at least 60 or at least 100 residues of the adipocyspin polypeptides and variants of the invention. Also provided by the invention are adipocyspin polypeptides that are modified, relative to the amino acid sequence of SEQ ID NO:1, in some manner; *e.g.*, truncated, mutated, derivatized; or fused to other sequences *(e.g.;* to form a fusion protein). Some adipocyspin polypeptides comprise insertions, deletions or substitutions of amino acid residues relative to SEQ ID NO:1. For example, some conservative amino acid substitutions can be made, *i.e.*, substitution of selected amino acids with different amino acids having similar structural characteristics, *e.g.*, net charge, hydrophobicity, and the like.

Typically, the adipocyspin variants are structurally and functionally similar to the adipocyspin allele having the sequence of SEQ. ID. NO:1. Structural similarity is indicated by, *e.g.*, substantial sequence identity (as defined above), or immunological cross-reactivity. Functional similarity is indicated by, *e.g.*, inhibition of conversion of preadipocytes into adipocytes.

In some embodiments, the adipocyspin polypeptide of the invention may be used as an immunogen (*e.g.*, to produce anti-adipocyspin antibodies). Typically, the immunogenic adipocyspin fragments of the invention comprise at least about 6 contiguous residues of SEQ ID NO:1, more often at least about 8, about 10, or about 12, or about 16 contiguous residues.

The substantially pure, isolated or recombinant adipocyspin polypeptides of the present invention can also be characterized by their ability to bind antibodies that are specifically immunoreactive with a polypeptide having the sequence shown in SEQ ID NO:1. Specific immunoreactivity is usually characterized by a specific binding affinity of an antibody for its ligand

(e.g., adipocyspin) of at least 10^7, 10^8, 10^9; or 10^{10} M^{-1}.

For many applications, it will also be desirable to provide adipocyspin polypeptides of the invention as labelled entities, *i.e.,* covalently attached or linked to a detectable label or group, or cross-linkable group, to facilitate identification, detection and quantification of the polypeptide in a given circumstance. These detectable groups can comprise a detectable polypeptide group, *e.g.,* an assayable enzyme or antibody epitope. Alternatively, the detectable group can be selected from a variety of other detectable groups or labels, such as radiolabels *(e.g.,* ^{125}I, ^{32}P, ^{35}S) or a chemiluminescent or fluorescent group. Similarly, the detectable group can be a substrate, cofactor, inhibitor or affinity ligand.

In addition, an adipocyspin polypeptide can be modified by substituting one or more amino acid residues with a D-amino acid of the same type *(e.g.,* D-lysine in place of L-lysine) to generate more stable peptides. Similarly, modification of the amino or carboxyl terminals can also be used to confer stabilizing properties upon the polypeptides of the invention, *e.g..* amidation of the carboxyl-terminus or acylation of the amino-terminus or pegylated derivatives.

B. Production and Isolation of Adipocyspin Polypeptides

The adipocyspin polypeptides of the present invention can be prepared using recombinant or synthetic methods, or can be isolated from natural cellular sources.

Suitable recombinant techniques for expressing adipocyspin polypeptides from the adipocyspin polynucleotides are disclosed *infra.* See also, Sambrook *et al.,* 1989, MOLECULAR CLONING: A LABORATORY MANUAL, (2nd ed.) Vols. 1-3, Cold Spring Harbor Laboratory, and in Ausubel, *supra.* Synthetic methods for synthesizing polypeptides such as adipocyspin polypeptides, variants, or fragments are described in Merrifield~ 1963) *Amer. Chem. Soc.* 85:2149-2456, Atherton *et al.,* 1989, SOLID PHASE PEPTIDE SYNTHESIS: A PRACTICAL APPROACH, IRL Press, and Merrifield, 1986, *Science* 232:341-347.

Isolation and purification of the adipocyspin polypeptides of the present invention can be carried out by methods that are generally well known in the art. These methods include, but are not limited to, ion exchange, hydrophobic interaction, HPLC or affinity chromatography, to achieve the desired purity. In one embodiment, adipocyspin polypeptides are purified using immunoaffinity chromatography. For example, antibodies raised against a adipocyspin polypeptide or immunogenic fragment thereof (*e.g.,* having a sequence or subsequence of SEQ ID NO:1) are coupled to a suitable solid support and contacted with a mixture of polypeptides containing the adipocyspin polypeptide (*e.g.,* a homogenate of adipose tissue) under conditions conducive to the association of this polypeptide with the antibody. Once the adipocyspin polypeptide is bound to the immobilized antibody, the solid support is washed to remove unbound material and/or nonspecifically bound

polypeptides. The desired polypeptide can then be eluted from the solid support in substantially pure form by, *e.g.*, a change in pH or salt concentration of the buffer.

C. Peptide Analogues and Peptide Mimetics of Adipocyspin

A1though primarily described in terms of "proteins" or "polypeptides," one of skill in the art will understand that structural analogues and derivatives of the above-described polypeptides, *e.g.*, peptidomimetics, and the like can be used as substitutes for adipocyspin , *e.g.*, as adipocyspin agonists, or, alternatively, as adipocyspin activity antagonists. Peptidomimetics, or peptide mimetics; are peptide analogues commonly used in the pharmaceutical industry as non-peptide drugs with properties *(e.g.*, a biological activity) analogous to those of the template peptide (Fauchere, 1986, *Adv. Drug Res.* 15:29; Evans *et al.*, 1987, *J. Med. Chem.* 30:1229). They are usually developed with the aid of computerized molecular modelling. Peptide mimetics that are structurally similar to therapeutically useful peptides can be used to produce an equivalent therapeutic effect. Peptide mimetics can have significant advantages over polypeptide embodiments, including, for example, more economical production and greater chemical stability.

D. Administration of Adipocyspin Polypeptide

IV. ANTIBODIES

The present invention provides antibodies that are specifically immunoreactive with human adipocyspin polypeptide. Accordingly, the antibodies of the invention will specifically recognize and bind polypeptides which have an amino acid sequence identical, or substantially identical, to the amino acid sequence of SEQ ID NO:1, or an immunogenic fragment thereof. The antibodies of the invention usually exhibit a specific binding affinity for adipocyspin of at least about 10^7, 10^8, 10^9, or 10^{10} M^{-1}.

The anti-adipocyspin antibodies of the invention have a variety of uses, *e.g.*, isolation of adipocyspin polypeptides *(e.g.*, by immunoaffinity chromatography), detection of adipocyspin polypeptides, and for inhibition of adipocyspin activity *(e.g., in vivo* or *in vitro).*

A. Production of Anti-Adipocyspin Antibodies

Anti-adipocyspin antibodies of the present invention can be made by a variety of means well known to those of skill in the art, *e.g.*, as described *supra.* As noted in Section I, *supra,* antibodies are broadly defined herein and specifically include fragments, chimeras and similar binding agents (*e.g.*, the products of phage display technology), that specifically binds an adipocyspin polypeptide or epitope. In one embodiment, the antibody is a recombinantly- prepared double-chain polypeptides containing antibody light and heavy chain variable domains sufficient for antigen-

specific binding, and at least a fragment of antibody light and heavy chain constant regions (*e.g.*, the C_H^1 domain of the heavy chain) sufficient to maintain association of the two polypeptides. In one embodiment, the antibody is a single chain antibody (sFv), for example comprising antibody light and heavy chain variable domains configured to bind adipocyspin.

Methods for production of polyclonal or monoclonal antibodies are well known in the art. *See, e.g.,* Coligan, CURRENT PROTOCOLS IN IMMUNOLOGY, Wiley/Greene, NY (1991); Stites *et al.* (eds.) BASIC AND CLINICAL IMMUNOLOGY (7th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein ("Stites"); Goding, MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE (2d ed.) Academic Press, New York, NY (1986); Kohler and Milstein, 1975t *Nature* 256:495-97; and Harlow and Lane. These techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors. *See,* Huse *et al.*, 1989, *Science* 246:1275-81; and Ward *et al.*, 1989, *Nature* 341:544-46.

For production of polyclonal antibodies, an appropriate target immune system is selected, typically a mouse or rabbit, but also including goats, sheep, cows, chickens, guinea pigs, monkeys and rats. The immunoglobulins produced by the host can be precipitated, isolated and purified by routine methods, including affinity purification. Substantially monospecific antibody populations can be produced by chromatographic purification of polyclonal sera.

For monoclonal antibodies appropriate animals will be selected and the desired immunization protocol followed. The antibodies of the invention may be of any isotype, *e.g.,* IgM, IgD, IgG IgA, and IgE, with IgG, IgA and IgM most referred. Preferred monoclonal anti-adipocyspin antibodies neutralize *(i.e.,* inhibit or block) one or more biological activities of adipocyspin. Such antibodies may be obtained by screening hybridoma supernatants for the desired 20 inhibitory activity. Monoclonal antibodies with affinities of 10^8 liters/mole, preferably 10^9 to 10^{10} or stronger, can be produced by the methods described below. The production of non-human monoclonal antibodies, *e.g.,* murine, lagomorpha, or equine, is well known and can be accomplished by, *e.g.,* immunizing a host animal with a preparation containing adipocyspin or fragments thereof. Antibody-producing cells obtained from the immunized animals are immortalized and screened, or screened first for the production of antibody which binds to the adipocyspin polypeptide and then immortalized.

Some anti-adipocyspin monoclonal antibodies of the present invention are humanized, human or chimeric, in order to reduce their potential antigenicity, without reducing their affinity for their target. Humanized antibodies have been described in the art. *See, e.g.,* 30 Queen, *et a/.,* 1989, *Proc. Nat'l Acad. Sci. USA* 86:10029; U.S. Patent Nos. 5,563,762; 5,693,761; 5,585,089 and 5,530,101. The human antibody sequences used for humanization can be the

sequences of naturally occurring human antibodies or can be consensus sequences of several human antibodies. *See* Kettleborough *et al.*, *Protein Engineering* 4:773 (1991); Kolbinger *et al.*, *Protein Engineering* 6:971 (1993). Humanized monoclonal antibodies against adipocyspin can also be produced using transgenic animals having elements of a human immune system *(see, e.g.,* U.S. Patent Nos. 5,569,825; 5,545,806; 5,693,762; 5,693;761; and 5,7124,350).

Useful anti-adipocyspin antibodies can also be produced using phage display technology (see, *e.g.,* Dower *et al.*, WO 91/17271 and McCafferty *et al.*, WO 92/01047). In these methods, libraries of phage are produced in which members display different antibodies on their outer surfaces. Antibodies are usually displayed as Fv or Fab fragments. Phage displaying antibodies with a desired specificity are selected by affinity enrichment to an adipocyspin polypeptide. Single chain antibodies can be produced using methods well known in the art (see, e.g., Colcher *et al.* (1999) *Ann. N Y Acad. Sci.* 880:263-80; Reiter (1996) *Clin. Cancer Res.* 2:245-52); U.S. pat. nos. 4,946,778; 5,260,203; 5,455,030; 5,518,889; and 5,534,621).

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to standard procedures of the art, including ammonium sulphate precipitation, affinity chromatography, gel electrophoresis and the like (see generally PROTEIN PURIFICATION: PRINCIPLES AND PRACTICE 3RD EDITION (Springer-Verlag, N.Y., 1994)).

An antibody (e.g. an anti-adipocyspin antibody), is substantially pure when at least about 80%, more often at least about 90%, even more often at least about 95%, most often at least about 99% or more of the polypeptide molecules present in a preparation specifically bind the Same antigen (e.g. adipocyspin polypeptide). For pharmaceutical uses, anti-adipocyspin immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity are most preferred.

B. Modification of Adipocyspin Antibodies

The antibodies of the present invention can be used with or without modification. Frequently, the antibodies will be labelled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. Such labels include those that are well known in the art, *e.g.,* radioactive, fluorescent, or bioactive *(e.g.,* enzymatic) labels. As labelled binding entities, the antibodies of the invention may be particularly useful in diagnostic applications.

Also encompassed by the invention are hybrid antibodies that share the specificity of antibodies against an adipocyspin polypeptide but are also capable of specific binding to a second moiety. In hybrid antibodies, one heavy and light chain pair is from one antibody and the other pair from an antibody raised against another epitope. This results in the property of multi-functional

valency, i.e., ability to bind at least two different epitopes simultaneously. Such hybrids can be formed by fusion of hybridomas producing the respective component antibodies, or by recombinant techniques.

C. Selection of Non-Cross Reacting Antibodies

In some embodiments, an anti-adipocyspin monoclonal or polyclonal antiserum is produced that is specifically immunoreactive with adipocyspin and is selected to have low crossreactivity against other homologous proteins such as cystatin, and any such crossreactivity is removed by immunoabsorption prior to use in the immunoassay. Methods for screening and characterizing monoclonal antibodies for specificity are well known in the art and are described generally in Harlow and Lane, supra.

In order to produce a polyclonal antisera (e.g., for use in an immunoassay). the protein of SEQ ID NO:1 a polyclonal antiserum is prepared using methods well known in the art such as those described supra. For example, recombinant protein may be produced in a mammalian cell line. An inbred strain of mice such as balb/c is immunized with the protein of SEQ ID NO:1 using a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, supra). Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used as an immunogen. Polyclonal sera are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against other human cysteine proteases (e.g., one or more of cystatin or known homologues) using a competitive binding immunoassay such as the one described in Harlow and Lane, supra, at pages 570-573. Immunoassays in the competitive binding format can be used for the crossreactivity determinations. For example, the protein of SEQ ID NO: 2 can be immobilized to a solid support. Proteins added to the assay compete with the binding of the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to the protein of SEQ ID NO:1. The percent cross reactivity for the above proteins is calculated. using standard calculations. Those antisera with less than 10% crossreactivity with each of the proteins listed above are selected and pooled. The cross-reacting antibodies are then removed from the pooled antisera by immunoabsorption with the above-listed proteins.

V. GENETIC ANALYSIS & PROFILES

In one aspect of the invention, expression of adipocyspin is monitored or determined for diagnosis of an individual with a disease state or a propensity toward a disease state associated with

adipocyspin regulation. In various embodiments, the disease state is.

The adipocyspin can be obtained from biological fluid such as serum or blood and analyzed by electrophoresis, HLPC, or mass spectrometry. The expression profile can be monitored by any of the methods disclosed herein or known in the art.

The monitoring can be accomplished by monitoring the level of adipocyspin in an individual. In one embodiment, the level or expression profiles in an individual are compared with a reference profile, where a statistically significant correlation in comparison with a reference profile is diagnostic of a condition.

VI. SCREENING AND IDENTIFICATION OF MODULATORS OF ADIPOCYSPIN ACTIVITY

The invention also provides assay methods which are capable of screening compounds that modulate the activity of the adipocyspin. Of particular interest are compounds that modulate the conversion of preadipocytes into adipocytes. This invention is particularly useful for screening compounds by using recombinant adipocyspin in a variety of drug screening techniques. Thus, the present invention includes methods to evaluate putative specific agonists or antagonists of adipocyspin function. Accordingly, the present invention is directed to the use of these compounds in the preparation and execution of screening assays for compounds which modulate the activity of the adipocyspin to prevent the conversion of NIH 3T3 L1 cells into adipocytes.

Preliminary screens can be conducted by screening for compounds capable of binding to the adipocyspin receptor, as at least some of the compounds so identified are likely adipocyspin modulators. The binding assays usually involve contacting an adipocyspin agonist such as adipocyspin protein with one or more test compounds and allowing sufficient time for the protein and test compounds to form a binding complex. Any binding complexes formed can be detected using any of a number of established analytical techniques. Protein binding assays include, but are not limited to, methods that measure co-precipitation, co-migration on non-denaturing SDS-polyacrylamide gels, and co- migration on Western blots. The adipocyspin protein utilized in such assays can be naturally expressed, cloned or synthesized adipocyspin. In one embodiment, the assay is a cell-based assay and cells are used which are stably or transiently transfected with a vector or expression cassette having a nucleic acid sequence which encodes the adipocyspin receptor. The cells are maintained under conditions appropriate for expression of the adipocyspin receptor and are contacted with a putative agent under conditions appropriate for binding to occur. Binding can be detected using standard techniques. For example, the extent of binding can be determined relative to a suitable control (for example, relative to background in the absence of a putative agent, or relative to a known ligand). Optionally, a cellular fraction, such as a membrane fraction, containing the receptor can be used in lieu of whole cells.

Detection of binding or complex formation can be detected directly or indirectly. For example, the putative agent can be labelled with a suitable label (e.g., fluorescent label, chemiluminescent label, isotope label, enzyme label, and the like) and binding can be determined by detection of the label. Specific and/or competitive binding can be assessed by competition or displacement studies, using unlabelled agent as a ligand (e.g., recombinant adipocyspin) as a competitor.

In other embodiments, binding inhibition assays can be used to evaluate the present compounds. In these assays, the compounds are evaluated as inhibitors of ligand binding using, for example, adipocyspin receptor expressed in NIH 3T3 L1 cells. In this embodiment, the adipocyspin receptor's contacted with a ligand such as adipocyspin, and a measure of ligand binding is made. The receptor is then contacted with a test agent in the presence of a ligand (e.g., recombinant adipocyspin) and a second measurement of binding is made. A reduction in the extent of ligand binding is indicative of inhibition of binding by the test agent. The binding inhibition assays can be carried out using whole cells which express the adipocyspin receptor, or a membrane fraction from cells which express adipocyspin receptor.

Certain screening methods involve screening for a compound that up-regulates (or, alternatively, inhibits) the expression or activity of adipocyspin. Such methods generally involve conducting cell-based assays in which test compounds are contacted with one or more cells expressing adipocyspin and then detecting a change (*e.g.*) increase or decrease) in adipocyspin expression (either transcript or translation product) or activity. Some assays are performed with cells that express endogenous adipocyspin (*e.g.*, adipocytes or NIH 3T3 L1 cells). Other expression assays are conducted with recombinant cells that express adipocyspin encoded in a suitable expression vector. In either case; adipocyspin expression can be detected in a number of different ways, as described herein. For example, the expression level of adipocyspin in a cell can be determined by probing the mRNA expressed in a cell with a probe that specifically hybridizes with a transcript (or complementary nucleic acid derived therefrom) of adipocyspin. Probing can be conducted by lysing the cells and conducting Northern blots or without lysing the cells using in situ-hybridization techniques (see above). Alternatively, adipocyspin protein can be detected using immunological methods in which a cell lysate is probe with antibodies that specifically bind to adipocyspin. Similarly, adipocyspin activity can be assayed by determining the rate of conversion of adipocyte precursors into adipocytes, for example pre-NIH 3T3 L1 cells into NIH 3T3 L1 adipocytes. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing the adipocyspin, *e.g.* the protein having the sequence of SEQ ID NO:1. Such cells, either in viable or fixed form, can be used for screening. A test compound can be assayed for binding or for competition with another ligand for

binding.

An example of a suitable assay, an adipocyspin protein (whether isolated or recombinant) is used which has at least one property) activity or functional characteristic of a human adipocyspin protein. The property can be suppression of conversion of preadipocytes into adipocytes.

In one embodiment, a composition containing an adipocyspin protein or variant thereof is maintained under conditions suitable for binding. The adipocyspin receptor is contacted with a putative agent (or a second composition containing at least one putative agent) to be tested and binding is detected or measured.

The level of expression or activity can be compared to a baseline value. Expression levels can also be determined for cells that do not express adipocyspin as a negative control. Such cells generally are otherwise substantially genetically the same as the test cells.

The test compounds, adipocyspin activity modulators or putative modulators and other compounds provided herein can also be evaluated using models of inflammation to assess the ability of the compound to exert an effect *in vivo*. Suitable models are described as follows:

Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period. See, *e.g.*, Fodor *et al.*, 1991, Science 251: 767-73, and other descriptions of chemical diversity libraries, which describe means for testing of binding affinity by a plurality of compounds. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified adipocyspin and/or cells expressing recombinant adipocyspin as provided by this invention.

The terms "upregulated" and "activation" when used in reference to the expression of a nucleic acid such as a gene (particularly UCP-2) refers to any process which results in an increase in production of a gene product. A gene product can be either RNA (including, but not limited to, mRNA) or protein. Accordingly, gene upregulation or activation includes those processes that increase transcription of a gene and/or translation of a mRNA.

Examples of gene upregulation or activation processes that increase transcription include, but are not limited to, those that facilitate formation of a transcription initiation complex, those that increase transcription initiation rate, those that increase transcription elongation rate, those that increase processivity of transcription and those that relieve transcriptional repression (by, for example. blocking the binding of a transcriptional repressor). Gene upregulation or activation can constitute, for example, inhibition of repression as well as stimulation of expression above an existing level. Examples of gene upregulation or activation processes that increase translation include those that increase translational initiation. those that increase translational elongation and those that increase mRNA stability. The level of gene expression, including the level of gene activation or upregulation, can be quantitated utilizing a number of established techniques

including, but not limited to, Northern-Blots. RNase protection assays (RPA), nucleic acid probe arrays, quantitative PCR (.g., the so-called TaqMan assays, dot blot assays and in- situ hybridization. These are described further infra. In general, gene upregulation or activation comprises any detectable increase in the production of a gene product, preferably an increase in production of a gene product by at least 50 to 100%. in other instances from about 2- to about 5-fold or any integer therebetween, in still other instances between about 5- and about 10-fold or any integer therebetween, sometimes between about 10- and about 20-fold or any integer therebetween, in other instances between about 20- and about 50-fold or any integer therebetween, in yet other instances between about 50- and about 100-fold or any integer therebetween, and in still other instances100-fold or more. The terms upregulated and gene activation can also mean that the observed activity relative to a baseline level is a statistically significant difference (i.e., increase).

As used herein a " baseline value" generally refers to a value (or ranges of values) against which an experimental or determined value (e.g., one determined for a patient sample as part of a diagnostic or prognostic test) is compared. Thus, in the case ofUCP-2 upregulation, the baseline value can be a value for UCP-2 activity or expression for a sample obtained from the same individual at a different time point. In other instances, the baseline value is a value determined for a control cell or individual, or a statistical value (e.g., an average or mean) established for a population of control cells or individuals. In the specific instance of UCP-2 upregulation, the control can be a cell, individual or populations thereof for which UCP-2 levels would not be expected to be upregulated. Thus, for instance, a control individual or control population can include healthy individuals, particularly those that have not suffered a stroke or those not susceptible to stroke. The population that serves as a control can vary in size, having as few as a single member, but potentially including tens, hundreds, thousands, tens of thousands or more individuals. W7zen the control is a large population, the baseline value can be a statistical value determined from individual values for each member or a value determined from the control population as an aggregate (e.g., a value measured for a population of cells within a well).

VII. METHODS OF TREATING ADIPOCYSPIN-MEDIATED CONDITIONS OR DISEASES

In yet another aspect, the present invention provides methods of treating adipocyspin-mediated conditions or diseases by administering to a subject having such a disease or condition, a therapeutically effective amount of an modulator of adipocyspin function, i.e., agonists (stimulators) and antagonists (inhibitors) of adipocyspin function or gene expression.

Diseases and conditions associated with altered adipocyspin expression or activity include obesity and conditions associated with increased adipocyte mass or fat mass. In one group of embodiments, diseases or conditions, including chronic diseases, can be treated with modulators of

adipocyspin function. These diseases or conditions include: obesity and conditions associated with increased fat mass, which may be treated by adipocyspin and adipocyspin agonists. Such modulators include small molecules agonists and antagonists of adipocyspin function or expression; antisense and ribozyme triplex polynucleotides; gene therapy, and the like. The methods and reagents of the invention may be used in treatment of animals such as mammals (*e.g.*, humans, non-human primates, cows, sheep, goats, horses, dogs, cats, rabbits; rats, mice) or in animal or *in vitro* (*e.g.*, cell-culture) models of human diseases.

VIII PHARMACEUTICAL COMPOSITIONS

The present invention further provides therapeutic compositions comprising agonists; antagonists, or ligands of adipocyspin, and methods of treating physiologic or pathologic conditions mediated by adipocyspin, including decreased adipocyspin activity.

Adipocyspin polypeptides, fragments thereof, sense and antisense polypeptides, anti-adipocyspin antibodies or binding fragments thereof, and antagonists or agonists (*e.g.* small molecule modulators) of adipocyspin activity; can be directly administered under sterile conditions to the host to be treated. However, while it is possible for the active ingredient to be administered alone; it is often preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. For example, the bioactive agent can be complexed with carrier proteins such as ovalbumin or serum albumin prior to their administration in order to enhance stability or pharmacological properties such as half-life. Furthermore; therapeutic formulations of this invention can be combined with or used in association with other chemotherapeutic or chemopreventive agents.

Therapeutic formulations can be prepared by any methods well known in the art of pharmacy. See, *e.g.)* Gilman et at. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon Press; and Remington, The Science of Practice and Pharmacy, 20th Edition. (2001) Mack Publishing Co., Easton, P.a.; Avis et al (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, N.Y.; Liebennan *et al.* (eds.) (1990) Pharmaceutical Dosage Forms: Tablets Dekker, N.Y.; and Liebennan et al (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, N.Y.

Depending on the disease to be treated and the subject's condition, the compounds of the present invention may be administered by oral, parenteral (*e.g.*, intramuscular, intraperitoneal, intravenous, ICV) intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray, nasal, vaginal, rectal. Sublingual, or topical routes of administration and may be

formulated, alone or together) in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles appropriate for each route of administration..

The pharmaceutical composition and method of the present invention may further comprise other therapeutically active compounds as noted herein which are usually applied in the treatment of the above mentioned pathological conditions.

In the treatment or prevention of conditions which require adipocyspin modulation, an appropriate dosage level will generally be about 0.001 to 100 mg per kg patient body weight per day which can be administered in single or multiple doses. Preferably, the dosage level will be about 0.01 to about 25 mg/kg per day; more preferably about 0.05 to about 10 mg/kg per day. A suitable dosage level may be about 0.01 to 25 mg/kg per day, about 0.05 to 10 mg/kg per day, or about 0.1 to 5 mg/kg per day. Within this range the dosage may be about 0.005 to about 0.05, 0.05 to 0.5 or 0.5 to 5 mg/kg per day. For oral administration, the compositions are preferably provided in the form of tablets containing from about 1 to 1000 milligrams of the active ingredient, particularly about 1, 5, 10, 15, 20, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 750, 800, 900, and 1000 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. The compounds may be administered on a regimen of 1 to 4 times per day, preferably once or twice per day.

It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination the severity of the particular condition and the host undergoing therapy.

The compounds of the present invention can be combined with other compounds having related utilities to prevent and treat inflammatory and immunoregulatory disorders and diseases, including asthma and allergic diseases, as well as autoimmune pathologies such as rheumatoid arthritis and atherosclerosis and those pathologies noted above.

IX. DETECTION AND QUANTIFICATION OF ADIPOCYSPIN POLYNUCLEOTIDES AND POLYPEPTIDES

The present invention provides a number of methods for detection and quantification of adipocyspin polypeptides and polynucleotides in biological samples. In one embodiment, expression or over expression of the adipocyspin gene product (*e.g.*, polypeptide or mRNA) is correlated with a disease or condition mediated by, or associated with the adipocyspin.

The biological samples can include, but are not limited to, a blood sample, serum, cells (including whole cells, cell fractions, cell extracts, and cultured cells or cell lines), tissues

(including tissues obtained by biopsy), body fluids *(e.g.,* urine, sputum, amniotic fluid, synovial fluid)) or from media (from cultured cells or cell lines), and the like. The methods of detecting or quantifying adipocyspin polynucleotides include, but are not limited to, amplification-based assays with signal amplification) hybridization based assays and combination amplification-hybridization assays. For detecting and quantifying adipocyspin polypeptides) an exemplary method is an immunoassay that utilizes an antibody or other binding agents that specifically binds to an adip polypeptide or epitope.

A. Assays for Adipocyspin Polynucleotides

1. Amplification-based methods

The polymerase chain reaction (PCR), or its variations, is an exemplary amplification-based assay. Examples of techniques sufficient to direct persons of skill through *in vitro* amplification methods are found in PCR TECHNOLOGY: PRINCIPLES AND APPLICATIONS FOR DNA AMPLIFICATION, H. Erlich, Ed. Freeman Press, New York. NY (1992); PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS, eds. Innis, Gelfland, Snisky, and White, Academic Press, San Diego, CA (1990). Other suitable target amplification methods include the ligase chain reaction (LCR; *e.g.,* Wu and Wallace, 1989, *Genomics* 4:560); strand displacement amplification (SDA; *e.g.,* Walker *et al.*, 1992, *Proc. Natl. A cad. Sci. U.S.A.* 89:392-396); the nucleic acid sequence based amplification (NASBA, Cangene, Mississauga, Ontario; *e.g.,* Compton, 1991, *Nature* 350:91), and the like. One useful variant of PCR is PCR ELISA *(e.g.,* Boehringer Mannheim Cat. No.1 636 111) in which digoxigenin-dUTP is incorporated into the PCR product. The PCR reaction mixture is denatured and hybridized with a biotin-labelled oligonucleotide designed to anneal to an internal sequence of the PCR product. The hybridization products are immobilized on streptavidin coated plates and detected using anti-digoxigenin antibodies.

2. Hybridization-based methods

A variety of methods for specific DNA and RNA measurement using polynucleotide hybridization techniques are known to those of skill in the art (see Sambrook, *supra).* Hybridization based assays refer to assays in which a polynucleotide probe is hybridized to a target polynucleotide. Usually the polynucleotide hybridization probes of the invention are entirely or substantially identical to a contiguous sequence of the adipocyspin nucleic acid sequence. Preferably, polynucleotide probes are at least about 10 bases, often at least about 20 bases, and sometimes at least about 200 bases or more in length. Methods of selecting polynucleotide probe sequences for use in polynucleotide hybridization are discussed in Sambrook, *supra.*

Polynucleotide hybridization formats are known to those skilled in tl1e art. In some formats, at least one of the target and probe is immobilized. The immobilized polynucleotide may be DNA, RNA, or another oligo- or poly-nucleotide, and may comprise natural or non-naturally occurring nucleotides, nucleotide analogues, or backbones. Such assays may be in any of several formats including: Southern, Northern, dot and slot blots, high-density polynucleotide or oligonucleotide arrays *(e.g.,* GeneChips TM Affymetrix), dip sticks, pins~ chips, or beads. All of these techniques are well known in the art and are the basis of many commercially available diagnostic kits. Hybridization techniques are generally described in Hames *et al.*, ed., NUCLEIC ACID HYBRIDIZATION, A PRACTICAL APPROACH IRL Press, (1985); Gall and Pardue *Proc. Nat'l. Acad. Sci., USA.,* 63: 378-383 (1969); and John et aI., *Nature,* 223: 582-587 (1969).

In one embodiment, *in situ* hybridization is used to detect adipocyspin sequences in a sample. *In situ* hybridization assays are well known and are generally described in Angerer *et al.*, METHODS ENZYMOL., 152: 649-660 (1987) and Ausubel, *supra.*

B. Adipocyspin Polypeptide Assays

In one embodiment, the adipocyspin polynucleotide is detected in a sample using an anti-adipocyspin antibody of the invention. A number of well established immunological binding assay are suitable for detecting and quantifying adipocyspin of the present invention. See, *e.g.,* U.S. Patents 4,366,241; 4,376,110; 4,517,288; and 4,837,168, and also METHODS IN CELL BIOLOGY VOLUME 37: ANTIBODIES IN CELL BIOLOGY, Asai, ed. Academic Press, Inc. New York (1993); BASIC AND CLINICAL IMMUNOLOGY 7th Edition, Stites & Terr, eds. (1991); Harlow, *supra [e.g.,* Chapter 14], and Ausubel, *supra, [e.g.,* Chapter 11], each of which is incorporated by reference in its entirety and for all purposes.

Immunoassays for detecting adipocyspin may be competitive or noncompetitive. Usually the adipocyspin gene product being assayed is detected directly or indirectly using a detectable label. The particular label or detectable group used in the assay is usually not a critical aspect of the invention, so long as it does not significantly interfere with the specific binding of the antibody or antibodies used in the assay. The label may be covalently attached to the capture agent *(e.g.,* an anti-adipocyspin antibody), or may be attached to a third moiety, such as another antibody, that specifically binds to the adipocyspin polypeptide at a different epitope than recognised by the capture agent.

1. Non-Competitive Immunoassay

Noncompetitive immunoassays are assays in which the amount of captured analyte (here, the adipocyspin polypeptide) is directly measured. One such assay is a two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on the

captured analyte. See, *e.g.,* Maddox *et al.*, 1983, J: *Exp. Med.,* 158:1211 for background information. In such an assay, the amount of adipocyspin in the sample is directly measured. For example, using a so-called "sandwich" assay, the capture agent (here, the anti- adipocyspin antibodies) can be bound directly to a solid substrate where they are immobilized.

These immobilized antibodies then capture polypeptide present in the test sample. Adipocyspin thus immobilized is then bound by a labelling agent, such as a second adipocyspin antibody bearing a label. Alternatively, the second adipocyspin antibody may lack a label, but it may, in turn, be bound by a labelled third antibody specific to antibodies of the species from which the second antibody is derived. The second can be modified with a detectable moiety, such as biotin, to which a third labelled molecule can specifically bind, such as enzyme-labelled streptavidin.

2. Competitive Immunoassay

In competitive assays, the amount of adipocyspin polypeptide present in the sample 10 is measured indirectly by measuring the amount of an added (exogenous) adipocyspin displaced (or competed away) from a capture agent *(e.g.,* anti-adipocyspin antibody) by the analyte present in the sample (*e.g.*, adipocyspin polypeptide). In one competitive assay, a known amount of adipocyspin is added to the sample and the sample is then contacted with a capture agent *(e.g.,* an anti-adipocyspin antibody) that specifically binds to adipocyspin. The amount of adipocyspin bound to the antibody is inversely proportional to the concentration of adipocyspin present in the sample.

Preferably, the antibody is immobilized on a solid substrate. The amount of adipocyspin bound to the antibody may be determined either by measuring the amount of adipocyspin present in an adipocyspin/antibody complex, or alternatively by measuring the amount of remaining uncomplexed adipocyspin. The amount of adipocyspin may be detected by providing a labelled adipocyspin molecule. For example, using the hapten inhibition assay, the analyte (in this case adipocyspin) is immobilized on a solid substrate. A known amount of anti-adipocyspin antibody is added to the sample, and the sample is then contacted with the immobilized adipocyspin. In this case, the amount of anti-adipocyspin antibody bound to the immobilized adipocyspin is inversely proportional to the amount of adipocyspin present in the sample. Again the amount of immobilized antibody may be detected by detecting either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. Detection may be direct where the antibody is labelled or indirect by the subsequent addition of a labelled moiety that specifically binds to the antibody as described above.

3. Other Assays

In addition to the competitive and non-competitive adipocyspin polypeptide immunoassays, the present invention also provides other assays for detection and quantification of adipocyspin polypeptides. For example, Western blot (immunoblot) analysis can be used to detect and quantify the presence of adipocyspin in the sample. The technique generally comprises separating sample polypeptides by gel electrophoresis on the basis of molecular weight, transferring the separated polypeptides to a suitable solid support (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with the antibodies that specifically bind adipocyspin. The anti-adipocyspin antibodies specifically bind to adipocyspin on the solid support. These antibodies may be directly labeled or alternatively maybe subsequently detected using labeled antibodies *(e.g.,* labelled sheep anti-mouse antibodies) that specifically bind to the anti-adipocyspin .

Furthermore, assays such as liposome immunoassays (LIA) are also encompassed by the present invention. LIA utilizes liposomes that are designed to bind specific molecules *(e.g.,* antibodies) and to release encapsulated reagents or markers. The released chemicals are then detected according to standard techniques (see, Monroe *et al.*; 1986, *Amer. Clin. Prod. Rev.* 5:34-41).

X.KITS

Reagents useful for the therapeutic and diagnostic (detection) methods of the invention are conveniently provided in kit form. Thus, the present invention encompasses kits that contain polypeptides, antibodies, and polynucleotides of the present invention.

In one embodiment, the kit comprises one or more of the following in a container: (1) adipocyspin polynucleotides (*e.g.*, oligonucleotide primers or probes corresponding to the adipocyspin cDNA sequence and capable of amplifying the target polynucleotides); (2) anti-adipocyspin antibodies; (3) adipocyspin polypeptides or fragments, optionally coated on a solid surface (such as a slide, multiple well plate, or test tube) (4) an adipocyspin polynucleotide *(e.g.,* for use as positive controls in assays), (5) and tubes. Instructions for carrying out the detection methods of the invention, and calibration curves can also be included.

In the following study, we report the identification of a novel adipocyte-secreted product which shares sequence homology with family members of cysteine protease inhibitors (we therefore name the protein as adipocyspin). The expression of adipocyspin was induced markedly during adipose conversion of 3T3-L1 cells. The mRNA abundance was also significantly increased in obesity states. Furthermore, treatment of 3T3 L1 cells with recombinant adipocyspin significantly inhibited the adipose conversion, indicating that adipocyspin is a negative regulator of adipogenesis.

The terms "allele" or "allelic sequence," as used herein, refer to a naturally-occurring alternative form of a gene encoding the adipocyspin polypeptide (i.e., a polynucleotide encoding an

adipocyspin polypeptide). Alleles result from mutations (i.e., changes in the nucleic acid sequence), and sometimes produce altered and/or differently regulated mRNAs or polypeptides whose structure and/or function may or may not be altered. Common mutational changes that give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides that may or may not affect the encoded amino acids. Each of these types of changes may occur alone, in combination with the others, or one or more times within a given gene, chromosome or other cellular polynucleotide. Any given gene may have no, one or many allelic forms. As used herein, the term "allele" refers to either or both a gene or an mRNA transcribed from the gene.

As used herein, the term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogues and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogues refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an alpha-carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulphoxide, methionine methyl sulphonium. Such analogues have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

The term "antisense sequences" refers to polynucleotides having sequence complementary to a RNA sequence. These terms specifically encompass nucleic acid sequences that bind to mRNA or portions thereof to block transcription of mRNA by ribosomes. Antisense methods are generally well known in the art (see, *e.g.*, PCT publication WO 94/12633, and Nielsen *et al.*, 1991, *Science* 254:1497; OLIGONUCLEOTIDES AND ANALOGUES, A PRACTICAL APPROACH, edited by F. Eckstein, IRL Press at Oxford University Press (1991); ANTISENSE RESEARCH AND APPLICATIONS (1993, CRC Press)).

The term "composition" as used herein is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

The term "conservative substitution," when describing a polypeptide, refers to a change in the amino acid composition of the polypeptide that does not substantially alter the activity of the polypeptide, i.e., substitution of amino acids with other amino acids having similar properties such that the substitutions of even critical amino acids does not substantially alter activity. Conservative substitution tables providing functionally similar amino acids are well known in the art. The

following six groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Serine (S), Threonine (1); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (1), Leucine (L), Methionine (M), Valine (V); and 6) Phenylalanine (F), T}Tyrosine (Y), Tryptophan (W)(see also, Creighton, 1984, *Proteins,* W.H. Freeman and Company).

In addition to the above-defined conservative substitutions, other modification of amino acid residues can result in "conservatively modified variants." For example, one may regard all charged amino acids as substitutions for each other whether they are positive or negative. In addition, conservatively modified variants can also result from individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids, *e.g.* often less than 5%, in an encoded sequence. Further, a conservatively modified variant can be made from a recombinant polypeptide by substituting a codon for an amino acid employed by the native or wild-type gene with a different codon for the same amino acid.

The terms "control elements" or "regulatory sequences" include enhancers, promoters, transcription terminators, origins of replication, chromosomal integration sequences, 5' and 3' untranslated regions, with which polypeptides or other biomolecules interact to carry out transcription and translation. For eukaryotic cells, the control sequences will include a promoter and preferably an enhancer, *e.g.,* derived from immunoglobulin genes, SV40, cytomegalovirus, and a polyadenylation sequence. and may include splice donor and acceptor sequences. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. When referring to adipocyspin, a promoter other than that naturally associated with the adipocyspin coding sequence can be referred to as a "heterologous" promoter.

As used herein, a "derivatized" polynucleotide, oligonucleotide, or nucleic acid refers to oligo- and polynucleotides that comprise a derivatized substituent. In some embodiments, the substituent is substantially non-interfering with respect to hybridization to complementary polynucleotides. Derivatized oligo- or polynucleotides that have been modified with appended chemical substituents *(e.g.,* by modification of an already synthesized oligo- or poly-nucleotide, or by incorporation of a modified base or backbone analogue during synthesis) may be introduced into a metabolically active eukaryotic cell to hybridize with an adipocyspin DNA, RNA, or protein where they produce an alteration or chemical modification to a local DNA, RNA, or protein. Alternatively, the derivatized oligo or polynucleotides may interact with and alter adipocyspin polypeptides, or proteins that interact with adipocyspin DNA or adipocyspin gene products, or alter or modulate expression or function of adipocyspin DNA, RNA or protein. Illustrative attached chemical substituents include: europium (III) texaphyrin, cross-linking agents, psoralen, metal

chelates *(e.g.,* iron/EDTA chelate for iron catalyzed cleavage), topoisomerases, endonucleases, exonucleases, ligases, phosphodiesterases, photodynamic porphyrins, chemotherapeutic drugs *(e.g.,* adriamycin, doxirubicin), intercalating agents, base-modification agents, immunoglobulin chains, and oligonucleotides. Iron/EDTA chelates are chemical substituents often used where local cleavage of a nucleic acid sequence is desired (Hertzberg *et al.*, 1982. *J. Am. Chem. Soc.* 104: 313; Hertzberg and Dervan,1984, *Biochemistry* 23: 3934; Taylor *et al.*, 1984, *Tetrahedron* 40: 457; Dervan, 1986, *Science* 232: 464). Illustrative attachment chemistries include: direct linkage, *e.g.,* via an appended reactive amino group (Corey and Schultz, 1988, *Science* 238: 1401, which is incorporated herein by reference) and other direct linkage chemistries, although streptavidin/biotin and digoxigenin/anti-digoxigenin antibody linkage methods can also be used. Methods for linking chemical substituents are provided in U.S. Patents 5,135,720, 5,093,245, and 5,055,556, which are incorporated herein by reference.

Other linkage chemistries may be used at the discretion of the practitioner.

As used herein, a "detectable label" has the ordinary meaning in the art and refers to an atom *(e.g.,* radionuclide), molecule *(e.g.,* fluorescein), or complex, that is or can be used to detect *(e.g.,* due to a physical or chemical property), indicate the presence of a molecule or to enable binding of another molecule to which it is covalently bound or otherwise associated. The term "label" also refers to covalently bound or otherwise associated molecules *(e.g.,* a biomolecule such as an enzyme) that act on a substrate to produce a detectable atom, molecule or complex. Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical, chemical means and the like.

The term "epitope" has its ordinary meaning of a site on an antigen recognized by an antibody. Epitopes are typically segments of amino acids which are a small portion of the whole polypeptide. Epitopes may be conformational *(i.e.,* discontinuous). That is, they may be formed from amino acids encoded by noncontiguous parts of a primary sequence that have been juxtaposed by protein folding.

The term "fusion protein," refers to a composite polypeptide, i.e.~ a single contiguous amino acid sequence, made up of two (or more) distinct, heterologous polypeptides which are not normally fused together in a single amino acid sequence. Thus, a fusion protein may include a single amino acid sequence that contains two entirely distinct amino acid sequences or two similar or identical polypeptide sequences, provided that these sequences are not normally found together in the same configuration in a single amino acid sequence found in nature. Fusion proteins may generally be prepared using either recombinant nucleic acid methods, i.e., as a result of transcription and translation of a recombinant gene fusion product, which fusion comprises a segment encoding a polypeptide of the invention and a segment encoding a heterologous polypeptide, or by chemical

synthesis methods well known in the art.

The term "gene product" refers to an RNA molecule transcribed from a gene, or a polypeptide encoded by the gene or translated from the RNA.

The term "high affinity" for an IgG antibody, as used herein, refers to an association constant (Ka) of at least about 10^6M^{-1}, preferably at least about 10^8M^{-1}, more preferably at least about 10^9M^{-1} or greater, *e.g.,* up to $1O^{12}M^{-1}$ or greater. However, "high affinity" binding can vary for other antibody isotypes.

The terms "immunogen" and "immunogenic" have their ordinary meaning in the art, *i.e.,* an immunogen is a molecule, such as a polypeptide or other antigen) that can elicit an adaptive immune response upon injection into a person or an animal.

The terms "modulator" and "modulation" of preadipocytes to adipocyte conversion activity, as used herein in its various forms, is intended to encompass antagonism, agonism, partial antagonism and/or partial agonism of the activity associated with a particular cell surface receptor, preferably the adipocyspin receptor. In various embodiments, "modulators" may inhibit or stimulate adipocyspin expression or activity. Such modulators include small molecules agonists and antagonists of adipocyspin function or expression; antisense and ribozyme triplex polynucleotides; gene therapy, and the like.

The terms "nucleic acid" and "polynucleotide" are used interchangeably and refer to deoxyribonucleotides or ribonucleotides and polymers thereof in either single-or double- stranded form. Unless specifically limited, the disclosure of a polynucleotide sequence is also intended to refer to the complementary sequence. As used herein, the term "polynucleotide" includes oligonucleotides.

The terms "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of approximately 7 nucleotides or greater, and as many as approximately 100 nucleotides, which can be used as a primer or probe. Oligonucleotides are often between about 10 and about 50 nucleotides in length) more often between about 12 and about 50 nucleotides, very often between about 15 and about 25 nucleotides.

The term "operably linked" refers to a functional relationship between two or more polynucleotide *(e.g.,* DNA) segments: for example, a promoter or enhancer is operably linked to a coding sequence if it stimulates the transcription of the sequence in an appropriate host cell or other expression system. Generally, sequences that are operably linked are contiguous, and in the case of a signal sequence both contiguous and in reading phase. However, enhancers need not be located in close proximity to the coding sequences whose transcription they enhance.

The terms "peptidomimetic" and "mimetic" refer to a synthetic chemical compound that has substantially the same structural and functional characteristics of the adipocyspin polypeptides of

the invention. Peptide analogues are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics" (Fauchere, J. Adv. Drug Res. 15:29 (1986); Veber and Freidinger TINS p. 392 (1985); and Evans et aI. J. Med. Chem. 30:1229 (1987), which are incorporated herein by reference). Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent or enhanced therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biological or pharmacological activity), such as a adipocyspin, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of, *e.g.*, -CH2NH-, -CH2S-, -CH2-CH2-, -CH=CH- (cis and trans), -COCH2-, -CH(OH)CH2-, and -CH2S0-. The mimetic can be either entirely composed of synthetic, non-natural analogues of amino acids, or, is a chimeric molecule of partly natural peptide amino acids and partly non-natural analogues of amino acids. The mimetic can also incorporate any amount of natural amino acid conservative substitutions as long as such substitutions also do not substantially alter the mimetic's structure and/or activity. For example, a mimetic composition is within the scope of the invention if it is capable of carrying out the binding or enzymatic activities of adipocyspin.

By "pharmaceutically acceptable" it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The term "polypeptide" is used interchangeably herein with the term "protein," and refers to a polymer composed of amino acid residues linked by amide linkages, including synthetic, naturally-occurring and non-naturally occurring analogues thereof (amino acids and linkages). Peptides are examples of polypeptides.

As used herein, a "probe," when used in the context of polynucleotides and antibodies, refers to a molecule that specifically binds another molecule. One example of a probe is a "nucleic acid probe," which can be a DNA, RNA, or other polynucleotide. Where a specific sequence for a nucleic acid probe is given, it is understood that the complementary strand is also identified and included. The complementary strand will work equally well in situations where the target is a double-stranded nucleic acid that specifically binds (*e.g.*, anneals or hybridizes) to a substantially complementary nucleic acid. Another example of a probe is an "antibody probe" that specifically binds to a corresponding antigen or epitope.

The term "recombinant" refers to a polynucleotide synthesized or otherwise manipulated *in vitro (e.g.,* "recombinant polynucleotide"), to methods of using recombinant polynucleotides to produce gene products in cells or other biological systems, or to a polypeptide ("recombinant protein") encoded by a recombinant polynucleotide. Thus, a "recombinant" polynucleotide is defined either by its method of production or its structure. In reference to its method of production,

the process is use of recombinant nucleic acid techniques, *e.g.*, involving human intervention in the nucleotide sequence, typically selection or production. Alternatively, it can be a polynucleotide made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature. Thus, for example, products made by transforming cells with any non-naturally occurring vector is encompassed, as are polynucleotides comprising sequence derived using any synthetic oligonucleotide process. Similarly, a "recombinant" polypeptide is one expressed from a recombinant polynucleotide.

The phrase "selectively hybridizing to" refers to a polynucleotide probe that hybridizes, duplexes or binds to a particular target DNA or RNA sequence when the target sequences are present in a preparation of total cellular DNA or RNA.

The phrase "specifically immunoreactive," or "specifically binds" when referring to the interaction between an antibody and a protein or polypeptide, refers to an antibody that specifically recognizes and binds with relatively high affinity to the protein of interest, *e.g.*, adipocyspin, such that this binding is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular polypeptide and do not bind in a significant amount to other polypeptides present in the sample. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular polypeptide. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a polypeptide. See, Harlow, 1988, ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Publications, New York (hereinafter, "Harlow"), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

As used herein, the "substantially sequence identity," or "substantially identical" (in the context of comparing two or more polypeptides or polynucleotides) refers to two or more sequences or subsequences that have at least 60%, preferably 80%, most preferably 90%, 95%, 98%, or 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. Two sequences (amino acid or nucleotide) can be compared over their full-length (*e.g.*, the length of the shorter of the two, if they are of substantially different lengths) or over a subsequence such as at least about 50, about 100, about 200, about 500 or about 1000 contiguous nucleotides or at least about 10, about 20, about 30, about 50 or about 100 contiguous amino acid residues. Substantially identical polypeptides, as used herein, preferably have a common functional activity (*e.g.*, biological activity).

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference

sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. BioI. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Natl. Acad. Sci. USA 85:2444 (1988),
by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally Ausubel *et al.*, Current Protocols In Molecular Biology, Greene Publishing and Wiley-Interscience, New York (supplemented through 2001). Each of these references and algorithms is incorporated by reference herein in its entirety. When using any of the aforementioned algorithms, the default parameters for "Window" length. gap penalty, etc., are used.

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, J. Mol. BioI. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul et aI, supra). These initial neighbourhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, *e.g.*, Karlin & Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST

algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the first polypeptide (*e.g.*, a polypeptide encoded by the first nucleic acid) is immunologically cross reactive with the second polypeptide (*e.g.*, a polypeptide encoded by the second nucleic acid). Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions.

Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions. Substantial identity exists when the segments will hybridize under stringent hybridization conditions to a strand, or its complement, typically using a sequence of at least about 50 contiguous nucleotides derived from the probe nucleotide sequences.

A difference is typically considered to be "statistically significant" if the probability of the observed difference occurring by chance (the p-value) is less than some predetermined level. As used herein a "statistically significant difference" refers to a p-value that is <0.05. preferably <0.01 and most preferably <0.001.

"Stringent hybridization conditions" refers to conditions in a range from about 5°C to about 20°C or 25°C below the melting temperature (Tm) of the target sequence and a probe with exact or nearly exact complementarity to the target. As used herein, the melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half-dissociated into single strands. Methods for calculating the Tm of nucleic acids are well known in the art (see, *e.g.*, Berger and Kimmel, 1987, Methods In Enzymology, Vol. 152: Guide To Molecular Cloning Techniques) San Diego: Academic Press) Inc. and Sambrook *et al.*; supra;(1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Vols. 1-3, Cold Spring Harbor Laboratory). As indicated by standard references, a simple estimate of the Tm value may be calculated by the equation: Tm = 81.5 + 0.41(% G + C), when a nucleic acid is in aqueous solution at 1 M NaCI (see *e.g.*, Anderson and Young, "Quantitative Filter Hybridization"' in Nucleic Acid Hybridization (1985)). Other references include more sophisticated computations which take structural as well as sequence characteristics into account for the calculation of Tm. The melting temperature of a hybrid (and thus the conditions for stringent hybridization) is affected by various factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA) RNA, base composition) present in solution or immobilized, and the like), and the

concentration of salts and other components (*e.g.*) the presence or absence of formamide) dextran sulfate, polyethylene glycol). The effects of these factors are well known and are discussed in standard references in the art, see *e.g.*) Sambrook, supra, and Ausubel, supra. Typically, stringent hybridization conditions are salt concentrations less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion at pH 7.0 to 8.3, and temperatures at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). As noted, stringent conditions may also be achieved with the addition of destabilizing agents such as formamide, in which case lower temperatures may be employed.

The terms "substantially pure" or "isolated," when referring to proteins and polypeptides, *e.g.*, adipocyspin, denote those polypeptides that are separated from proteins or other contaminants with which they are naturally associated. A protein or polypeptide is considered substantially pure when that protein makes up greater than about 50% of the total protein content of the composition containing that protein, and typically, greater than about 60% of the total protein content. More typically, a substantially pure or isolated protein or polypeptide will make up at least 75%, more preferably, at least 90%, of the total protein. Preferably, the protein will make up greater than about 90%, and more preferably, greater than about 95% of the total protein in the composition. When referring to polynucleotides, the terms "substantially pure" or "isolated" generally refer to the polynucleotide separated from contaminants with which it is generally associated, *e.g.*, lipids, proteins and other polynucleotides. The substantially pure or isolated polynucleotides of the present invention will be greater than about 50% pure. Typically, these polynucleotides will be more than about 60% pure, more typically, from about 75% to about 90% pure and preferably from about 95% to about 98% pure.

The term "therapeutically effective amount" means the amount of the subject compound that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

As used herein, a "biological effect" refers to the conversion of preadipocytes to adipocytes.

A better understanding of the invention will be gained by reference to the following experimental section the following experiments are illustrative of the present invention and are not intended to limit the invention in any way.

EXAMPLE 1

Sets out the experimental procuedures utilised to differentiate #T#-LI cells and subsequent concentration of the resultant proteing from the cell culture medium.

3T3-L1 cells were maintained as subconfluent cultures in DMEM that was supplemented with 10% fetal calf serum. For differentiation to occur the cells were seeded onto 150

mm plates and allowed to reach 100% confluence and induced one-day post confluence with the above medium containing 0.25 μM dexamethasone, 0.5 mM IBMX and 10 μg/ml insulin for 2 days. This was then followed by incubation with 10 μg/ml insulin for 2 days. The cells were then maintained in DMEM with 10% fetal calf serum for another 4 days.

To harvest the proteins secreted from the adipocytes the cells 8 days after differentiation, were washed three times with PBS, and then incubated with serum-free medium for another 4 hours. The medium was then collected, centrifuged at 3,000 × g for 10 min, filtered through 0.20 μm filter, and then concentrated and desalted using a concentrator with MWCO of 5000 Da (Vivascience Ltd, Gloucestershire, UK). The proteins were then quantitated using BCA reagent, and stored at -80 °C until use.

The proteins secreted from either adipocytes or 3T3 L1 preadipocytes were separated by two dimensional gel electrophovesis as described previously, using Immobiline DryStrips with a pH range of 6-11. The separated proteins were stained with either silver or Coomassie Brilliant Blue R250 (CBB). The differentially secreted proteins were identified by Melanine 2 software.

Proteins of interest, that were separated by 2-DE gels, were excised and gel pieces were subjected to in-gel trypsin digestion as described previously. The extracted tryptic peptide mixtures were fractionated by RP HPLC on a Jupiter 5μ C18 column (250 × 2.00 mm, Phenomenex). The pre-warmed column (37 °C) was washed for 7 min with 0.1% trifluoroacetic acid (v/v) followed by elution using a 50 min linear gradient from 8% to 36% of acetonitrile at the flow rate of 200 μl/min. The well-separated fractions were chosen for amino acid sequencing using the Edman degradation method with a Perkin-Elmer (Procise, Model 492) protein sequencer.

Cloning and mammalian expression of adipocyspin— Total RNA was purified from mouse 3T3-L1 adipocytes or human fat pads using TRIZOL reagent according to the manufacturer's instructions. The oligo-dT-primed cDNA from the total RNA was used as a template for PCR cloning. The full-length cDNAs of mouse and human adipocyspin were inserted into pGEMT-easy vector (Promega) for DNA sequence verification.

The vector for mammalian expression of mouse adipocyspin was generated by cDNA amplification using 5'GCCCGCGGATCCATGCTACTGTTGCAAGCTCT3' as the sense primer and

5'GGCCGCGAATTCTCACTTGTCATCGTCGTCCTTGTAGTCGTTGGTATCATGGTAGAG3' as the antisense primer. Following digestion with BamHI/EcoRI, the fragment was inserted into pcDNA3.1 vector to produce pcDNA-Adipocyspin-F, which encodes full-length adipocyspin with FLAG epitope tagged at its C-terminus. This mammalian expression vector was transfected into COS-7 cells using FuGENE 6 transfection reagent, and the cells were allowed to secrete

adipocyspin into serum free medium for 48 hr. The medium was then harvested and the cell debris removed by centrifugation at 3,000 × g for 10 min followed by filtration through a 0.2 μm filter. The filtered medium was concentrated using a Vivian concentrator with MWCO of 5000 Da, as described above. FLAG tagged adipocyspin was purified using anti-FLAG M2 affinity Gel and eluted with 150 μg/ml of FLAG peptide, per manufacturer's instructions (Sigma).

Northern blot and Western blot analysis— 10 μg of total RNA purified from either 3T3 L1 cells or mouse adipose tissue was separated 1.2% formaldehyde-denaturing agarose gel and transferred to Nylon membranes. Hybridization was carried out as described previously; using ^{32}P labelled full-length adipocyspin, adiponectin, and PPAR γ or GLUT4 cDNAs as a probe. The membranes were visualized and analysed using a phosphorimager. Western blot analysis was performed as described previously.

EXAMPLE 2

This example characterized adipocyspin, a novel secretory protein differentially expressed during adipocyte conversion.

To identify differentially expressed and secreted proteins during the adipocyte conversion, the proteins from culture medium of 3T3 L1 preadipocytes or adipocytes were separated by two dimensional gel electrophoresis. This analysis showed that a protein with apparent MW of 16 kDa and pI value of 9.3 was preferentially present in the adipocytes and not in preadipocytes (Figure1.). To identify the nature of this protein, the protein "spots" (see Figure 1) were excised from multiple preparative gels and then subjected to in gel trypsin digestion. The tryptic peptide mixtures were fractionated by RP HPLC and the well-separated fractions were subjected to amino acid sequencing (Figure 2). The amino acid sequences derived from the four tryptic peptides can not be assigned to any known proteins. However, tBLASTn searching of the nucleic acid database at the National Center for Biotechnology Information revealed that these amino acid sequences perfectly match with a hypothetical protein translated from an expressed sequence tag (EST) sequence from RIKEN full-length enriched murine adult cDNA library (gene accession number: AK002298). RT PCR analysis confirmed that this gene was indeed expressed in 3T3L1 adipocytes.

The hypothetical reading frame of this cDNA sequence encodes a protein of 162 amino acid residues (Figure 3). One hydrophobic stretch is predicated by a Kyte-Doolittle plot located within the first 17 amino-terminal residues and is characteristic of a signal sequence. Homology searching revealed that N-terminal half of this protein bears strong similarity to a family of proteins with cystatin-like domain such as cystatin C (Figure 3B). Cystatins are a family of cysteine protease inhibitors and many of them are secretory proteins. Given the potential role as a cysteine protease

inhibitor, we named the gene protein above as adipocyspin. The COOH-terminal half of adipocyspin displays little homology to any known proteins. The predicted molecular mass and pI value of adipocyspin (excluding the putative secretory signal) is 16548.23 Da and 9.36, which perfectly matches to the value that we observed during 2DE separation (Fig. 2).

EXAMPLE 3

To further confirm that adipocyspin is a secretory protein, a FLAG epitope tagged adipocyspin construct was introduced into COS 7 cells by transient transfection and the protein was detected in the conditioned medium by immunoblotting.

This analysis showed that adipocyspin can be readily detected in the culture medium (Fig. 4). On the other hand, β tubulin, an cytoplasmic protein, was hardly detectable, suggesting that adipocyspin in the cell culture medium is not due to the cell lysis.

EXAMPLE 4

This example assessed the Differentiation-dependent expression of adipocyspin mRNA by examining the time course of adipocyspin mRNA expression during the adipose conversion of 3T3 L1 preadipocytes by Northern blot analysis. As shown in Figure 5, adipocyspin mRNA correlate well with cell differentiation and changes in cell morphology (round shape and appearance of intracellular lipid droplets). The adipocyspin mRNA with the mass of approximately 800 bp started to appear as early as day 2 following induction of adipose conversion, and reached to the maximum at day 8. The expression kinetics of adipocyspin paralleled those of aP2, and slightly earlier than adiponectin, a protein exclusively expressed in adipocytes.

Thus, our results demonstrated a simultaneous appearance of adipocyspin mRNA expression and adipocyte phenotype.

EXAMPLE 5

This example assed the altered expression of adipocyspin in obesity states where the modulation of gene expression in the states of obesity may provide valuable clues for functional relevance of the protein of interest in metabolic disease states.

We observed a consistent three to four-fold increase of adipocyspin mRNA in obese (ob/ob) mice relative to their cogenic lean controls (Figure 6). This result is in sharp contrast with the decreased expression of adiponectin gene in ob/ob mice. Altered expression of adipocyspin in obesity suggests that this protein might contribute to some of the pathophysiological features of such states. Alternatively, adipocyspin mRNA levels may be controlled by genetic defects specific for this model, i.e., leptin.

EXAMPLE 6

This example assessed whether adipocyspin inhibits adipocyte differentiation proteolysis has been shown to play an important role in adipose conversion. Given that adipocyspin contains a potential cysteine protease inhibitor domain, we tested whether the protein is involved in adipocyte differentiation.

To this end, COOH-terminal FLAG-tagged adipocyspin was purified from culture medium of transiently transfected COS 7 cells and then added to 3T3 L1 cells. In the absence of adipocyspin, over 80% of 3T3 L1 cells were differentiated into lipid-laden adipocytes as shown in oil red O staining see A of Figure 7. In the cells treated with 20 μg/ml adipocyspin, only sporadic occurrence of lipid laden cells (less than 1 in 50) was observed. Similarly, the expression of adipocyte markers, PPAR γ and GLUT4, was also decreased by over 70% when the cells were treated with adipocyspin (Fig. 8). These results indicate that adipocyspin can block adipose conversion.

* * *

All patents, publications, scientific articles, web sites, and other documents and materials referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced document and material is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such patents, publications, scientific articles, web sites, electronically available information, and other referenced materials or documents.

The written description portion of this patent includes all claims. Furthermore, all claims, including all original claims as well as all claims from any and all priority documents, are hereby incorporated by reference in their entirety into the written description portion of the specification, and Applicants reserve the right to physically incorporate into the written description or any other portion of the application, any and all such claims. Thus, for example, under no circumstances may the patent be interpreted as allegedly not providing a written description for a claim on the assertion that the precise wording of the claim is not set forth *in haec verba* in written description portion of the patent.

The claims will be interpreted according to law. However, and notwithstanding the alleged or perceived ease or difficulty of interpreting any claim or portion thereof, under no circumstances may any adjustment or amendment of a claim or any portion thereof during prosecution of the application or applications leading to this patent be interpreted as having

forfeited any right to any and all equivalents thereof that do not form a part of the prior art.

All of the features disclosed in this specification may be combined in any combination. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Thus, from the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Other aspects, advantages, and modifications are within the scope of the following claims and the present invention is not limited except as by the appended claims.

The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. Thus, for example, in each instance herein, in embodiments or examples of the present invention, the terms "comprising", "including", "containing", *etc.* are to be read expansively and without limitation. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims.

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by various embodiments and/or preferred embodiments and optional features, any and all modifications and variations of the concepts herein disclosed that may be resorted to by those skilled in the art are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the

invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

It is also to be understood that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise, the term " X and/or Y" means "X" or "Y" or both "X" and "Y", and the letter "s" following a noun designates both the plural and singular forms of that noun. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

Other embodiments are within the following claims. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically and/or expressly disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

CLAIMS

What is claimed is:

1. A polynucleotide comprising a sequence encoding a polypeptide that has an activity and which is:
(a) a polynucleotide encoding the sequence of SEQ ID NO:1; or
(b) a polynucleotide which hybridizes under stringent conditions to (a); or
(c) a polynucleotide sequence which is degenerate as a result of the genetic code to the sequences defined in (a) or (b).

2. The polynucleotide of claim 1 that is operably linked to a promoter.

3. A recombinant vector comprising the polynucleotide of claim 2.

4. The vector of claim 3 that is an expression vector.

5. A cell transfected with a vector comprising the vector of claim 3.

6. The cell of claim 5 that is a eukaryotic cell or a mammalian cell.

7. A method for producing an adipocyspin protein; peptide or fusion protein comprising culturing the cell of claim 5 under conditions in which the polynucleotide is expressed.

8. An isolated or recombinant adipocyspin polypeptide of SEQ ID NO:1 or a fragment thereof; wherein said polypeptide demonstrates the activity of suppressing the conversion of preadipocytes to adipocytes.

9. The adipocyspin polypeptide or fragment thereof of claim 8 wherein the adipocytes are 3T3 L1 cells.

10. The polypeptide or fragment of claim 1, wherein the polypeptide has an 30 amino acid sequence that is at least 90% identical to SEQ ID NO:1.

11. The polypeptide or fragment of claim 8, that specifically binds to an antibody generated against

a polypeptide of SEQ ID NO.1.

12. The polypeptide of claim 8 that has an amino acid sequence identical to SEQ ID NO:1, or an immunogenic fragment thereof.

13. A fusion protein comprising a polypeptide of claim 8.

14. An isolated polynucleotide that encodes the polypeptide of claim 8 or a fragment thereof.

15. A polynucleotide primer, probe, antisense oligonucleotide or ribozyme comprising at least 15 contiguous bases identical or exactly complementary to those encoding SEQ ID NO:1.

16. An antibody, or fragment thereof, wherein the antibody or antibody fragment specifically binds to the polypeptide of claim 8.

17. The antibody of claim 16 that is monoclonal.

18. The antibody of claim 17, wherein the antibody binds with an affinity of at least about 10^8 M^{-1}.

19. An isolated cell capable of secreting the antibody of claim 17.

20. A hybridoma capable of secreting the antibody of claim 17.

21. A method of detecting an adipocyspin gene product in a sample comprising:
a) contacting the sample with a probe that specifically binds the gene product wherein the probe and the gene product form a complex, and detecting the formation of the complex; or,
b) specifically amplifying the gene product in the biological sample, wherein said gene product is a polynucleotide, and detecting the amplification product; wherein the formation of the complex or presence of the amplification product is correlated with the presence of the adiponectin gene product in the biological sample.

22. The method of claim 21, wherein the gene product is a polypeptide.

23. The method of claim 21, wherein the probe is an antibody.

24. The method of claim 21, wherein gene product is an RNA and the probe is a polynucleotide.

25. A method of amplifying an adipocyspin polynucleotide in a sample comprising
(a) adding reagents sufficient for a polymerase chain reaction and at least two different primers to the sample, wherein each of said primers comprise at least 10 contiguous nucleotides identical or exactly complementary to SEQ. ID NO:1; or,
(b) adding reagents sufficient for a ligase chain reaction and at least two different oligomers to the sample, wherein each of said primers comprise at least 10 contiguous nucleotides identical or exactly complementary to those encoding SEQ. ID NO: 1.

26. A method of identifying a modulator of adiponectin activity comprising contacting a cell expressing a recombinant polynucleotide of claim 1 and a test compound and assaying for a biological effect that occurs in the presence but not absence of the test compound, wherein a test compound that induces a biological effect is identified as a modulator of adipocyspin activity.

27. The method of claim 26, wherein the biological effect is suppression of conversion of preadipocytes to adipocytes.

28. A process for providing a pharmaceutical composition, comprising effecting the steps of a method of claim 26 and thereafter formulating a modulator of adipocyspin activity for pharmaceutical use.

29. A method of treating an adipocyspin-mediated condition in a mammal comprising administering an agent that modulates the activity or expression of adipocyspin in a cell or tissue in the mammal.

30. The method of claim 29 wherein the agent is an agent that inhibits the conversion of preadipocytes to adipocytes.

31. The method of claim 33 wherein the condition associated with insufficient action of adipocyspin is obesity.

33. A method for treating diabetes, obesity, or a cardiovascular disorder, comprising administration of a therapeutically effective amount of a compound which was identified by the method of claim 8.

34. An adipocyspin polypeptide that is recombinant, isolated, purified, or synthesized.

35. An adipocyspin polypeptide as claimed in claim 34 wherein said adipocyspin polypeptide is human adipocyspin.

36. An adipocyspin polypeptide as claimed in claim 34 wherein at least one pair of the cysteine residues are joined in an intramolecular disulphide bond.

37. An adipocyspin polypeptide as claimed in claim 34 wherein two pairs of the cysteine residues are joined in intramolecular disulphide bonds.

38. An adipocyspin polypeptide as claimed in claim 34 wherein all three pairs of the cysteine residues are joined in intramolecular disulphide bonds.

39. An adipocyspin polypeptide as claimed in claim 34 formulated with one or more of the group consisting of pharmaceutically acceptable excipients, co-actives or diluents so as to be suitable for administration to a mammalian patient.

40. Human adipocyspin formulated with one or more of the group consisting of pharmaceutically acceptable excipients, co-actives or diluents so as to be suitable for administration to a mammalian patient.

41. A pharmaceutical dosage unit containing an adipocyspin polypeptide wherein at least one pair of the cysteine residues are joined in an intramolecular disulphide bond.

42. A composition comprising an adipocyspin polypeptide wherein the adipocyspin polypeptide is recombinant, isolated, purified, or synthesized.

43. A composition according to claim 42 wherein the adipocyspin polypeptide is human adipocyspin.

44. A composition according to claim 42 or claim 43 formulated with or without other pharmaceutically acceptable excipients, co-actives, diluents or the like so as to be suitable for administration to mammalian patients.

45. The composition according to claim 44 wherein one of the pairs of cysteine residues is joined in an intramolecular disulphide bone.

46. The composition according to claim 44 wherein two of the pairs of cysteine residues is joined in an intramolecular disulphide bone.

47. The composition according to claim 44 wherein three of the pairs of cysteine residues is joined in an intramolecular disulphide bone.

48. The composition according to claim 44 wherein the adipocyspin polypeptide has the sequence of a naturally occurring mammalian adipocyspin polypeptide.

49. The composition according to claim 45 wherein the adipocyspin polypeptide has the sequence of human adipocyspin

50. The composition according to claim 48 wherein the administration of the composition to a mammal decreases the amount of adipose tissue in said mammal.

51. The composition according to claim 48 wherein the administration of the composition to a mammal decreases the fat mass of said mammal.

52. The composition according to claim 48 effective to elicit a plasma adipocyspin polypeptide concentration of between 1 microg/mL and 20 microg/mL.

53. The composition according to claim 48 effective to elicit a plasma adipocyspin polypeptide concentration of between 1.9 microg/mL and 17 microg/mL.

54. An adipocyspin polypeptide as claimed in claim 34 which is at least about 50% pure.

55. An adipocyspin polypeptide as claimed in claim 34 which is at least about 80% pure.

56. An adipocyspin polypeptide as claimed in claim 34 which is at least about 90% pure.

57. An adipocyspin polypeptide as claimed in claim 34 which is at least about 95% pure.

58. An adipocyspin polypeptide as claimed in claim 34 which is at least about 99% pure.

59. An adipocyspin polypeptide as claimed in claim 35 which is at least about 50% pure.

60. An adipocyspin polypeptide as claimed in claim 35 which is at least about 80% pure.

61. An adipocyspin polypeptide as claimed in claim 35 which is at least about 90% pure.

62. An adipocyspin polypeptide as claimed in claim 35 which is at least about 95% pure.

63. An adipocyspin polypeptide as claimed in claim 35 which is at least about 99% pure.

64. An adipocyspin polypeptide as claimed in claim 40 which is at least about 50% pure.

65. An adipocyspin polypeptide as claimed in claim 40 which is at least about 80% pure.

66. An adipocyspin polypeptide as claimed in claim 40 which is at least about 90% pure.

67. An adipocyspin polypeptide as claimed in claim 40 which is at least about 95% pure.

68. An adipocyspin polypeptide as claimed in claim 40 which is at least about 99% pure.

69. A method of diagnosing in an individual the presence of, or pre-disposition towards developing, a disease state comprising determining the level of an adipocyspin polypeptide in the individual and comparing the level with a level characteristic of an individual who is not suffering from the disease state, wherein a difference in levels is indicative of the presence of or propensity to develop the disease.

70. The method according to claim 69 wherein any one or more of the adipocyspin polypeptide isoforms utilized is has one, two or three pairs of its cysteine residues joined in intramolecular disulphide bonds.

71. The method according to claim 69 wherein any one or more of the adipocyspin polypeptide isoforms utilized is human adipocyspin.

72. The method according to claim 69 or claim 70 wherein the individual is a human.

73. The method according to claim 69 wherein the disease state is hyperglycemia, insulin resistance, metabolic syndromes associated with insulin resistance, Type 2 diabetes mellitus, or obesity, metabolic syndromes including hypertension, artherosclerosis, coronary heart disease, ischemic heart disease, or polycystic ovary syndrome.

74. The method according to claim 69 wherein the adipocyspin polypeptide is obtained from a biological sample.

75. The method according to claim 69 wherein the assessment method utilises electrophoresis, HLPC, or mass spectrometry.

76. The method according to claim 74 wherein the levels or expression patterns are quantitated or assessed using antibodies specific to glycosylated adipocyspin polypeptide isoforms.

77. A method of diagnosing in an individual the presence of, or pre-disposition towards developing, a disease state comprising determining the level of a specific adipocyspin polypeptide isoform in the individual and comparing the expression profile with a expression profile characteristic of an individual who is suffering from the disease state, wherein a similarity in expression profiles is indicative of the presence of or propensity to develop the disease.

78. The method according to claim 74 wherein any one or more of the adipocyspin polypeptide isoforms utilized is an adipocyspin polypeptide as claimed in claim 8.

79. The method according to claim 74 wherein any one or more of the adipocyspin polypeptide isoforms utilized is human adipocyspin.

80. The method according to claim 77 or claim 78 wherein the individual is a human.

81. The method according to claim 80 wherein the disease state is hyperglycemia, insulin resistance, metabolic syndromes associated with insulin resistance, Type 2 diabetes mellitus, or obesity, metabolic syndromes including hypertension, artherosclerosis, coronary heart disease, ischemic heart disease, or polycystic ovary syndrome.

82. The method according to claim 80 wherein the adipocyspin polypeptide is obtained from a biological sample.

83. The method according to claim 80 wherein the levels or expression patterns are obtained by quantitating or assessing the expression levels of an adipocyspin peptide.

84. The method according to claim 77 wherein the assessment method utilises electrophoresis, HLPC, or mass spectrometry.

85. The method according to claim 80 wherein the levels or expression patterns are quantitated or assessed using antibodies specific to adipocyspin polypeptide isoforms.

86. A method for treating a disease state associated with adipocyspin polypeptide regulation or aberrant insulin sensitivity comprising administering with or without pharmaceutically acceptable excipients, co-actives, diluents or the like an effective amount of adipocyspin polypeptide.

87. The method according to claim 86 wherein the disease state is hyperglycemia, insulin resistance, metabolic syndromes associated with insulin resistance, Type 2 diabetes mellitus, or obesity, metabolic syndromes including hypertension, artherosclerosis, coronary heart disease, ischemic heart disease, or polycystic ovary syndrome.

88. The method according to claim 86 wherein the adipocyspin polypeptide is human adipocyspin.

89. The method according to claim 86 wherein the adipocyspin polypeptide is selected from one or more of the following;

i) an adipocyspin polypeptide wherein at least one of the cysteine residues corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues,

ii) an adipocyspin polypeptide as defined in i) wherein two pairs of the cysteine residues are joined in intramolecular disulphide bonds,

iii) an adipocyspin polypeptide wherein three pairs of the cysteine residues are joined in disulphide bonds, and

iv) an adipocyspin polypeptide as defined in iii) wherein the said adipocyspin polypeptide is human adipocyspin .

90. A method for treating a disease state associated with adipocyspin polypeptide regulation or aberrant insulin sensitivity comprising administering with or without pharmaceutically acceptable excipients, co-actives, diluents or the like an effective amount of the composition of claim 89.

91. The method according to claim 89 wherein the disease state is hyperglycemia, insulin resistance, metabolic syndromes associated with insulin resistance, Type 2 diabetes mellitus, or obesity, metabolic syndromes including hypertension, artherosclerosis, coronary heart disease, ischemic heart disease, or polycystic ovary syndrome.

92. The use of an adipocyspin polypeptide (optionally with pharmaceutically acceptable excipients, co-actives, diluents and containment vessels) in the preparation of a pharmaceutical composition or medicament or dosage unit useful in a mammalian patient:
i) in the treatment of a disease state associated with adipocyspin polypeptide regulation; or
ii)to enhance the effects of insulin; or
iii)inhibit obesity or states associated with increased fat mass.

93. The use according to claim 90 wherein the adipocyspin polypeptide is human adipocyspin.

94. The use according to claim 90 wherein the adipocyspin polypeptide is selected from one or more of the following;

i) an adipocyspin polypeptide wherein at least one of the cysteine residues corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues,

ii) an adipocyspin polypeptide as defined in i) wherein two pairs of the cysteine residues are joined in intramolecular disulphide bonds,

iii) an adipocyspin polypeptide wherein three pairs of the cysteine residues are joined in disulphide bonds

iv) an adipocyspin polypeptide as defined in iii) wherein the said adipocyspin polypeptide is human adipocyspin

95. An article of manufacture comprising or including a vessel or delivery unit containing at least disulphide-bonded adipocyspin polypeptide and instructions for use of the disulphide-bonded adipocyspin polypeptide effective for use in a mammalian patient:

i) in the treatment of a disease state associated with adipocyspin polypeptide regulation; or

ii) to enhance the effects of insulin; or iii)to inhibit obesity or states associated with increased fat mass.

96. An article according to claim 95 wherein the disulphide-bonded adipocyspin polypeptide is human adipocyspin.

97. An article according to claim 95 wherein the adipocyspin polypeptide is selected from one or more of the following;

i) an adipocyspin polypeptide wherein at least one of the cysteine residues corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues,

ii) an adipocyspin polypeptide as defined in i) wherein two pairs of the cysteine residues are joined in intramolecular disulphide bonds,

iii) an adipocyspin polypeptide wherein three pairs of the cysteine residues are joined in disulphide bonds

iv) an adipocyspin polypeptide as defined in iii) wherein the said adipocyspin polypeptide is human adipocyspin

98. An article according to claim 95 wherein each of the cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues.

99. A formulation or dosage form capable of delivery of an effective amount of disulphide-bonded adipocyspin polypeptide when administered or self administered to a human being or other mammal sufficient to be effective for use in the treatment of a disease state associated with adipocyspin polypeptide regulation in a mammalian patient.

100. A formulation or dosage form of claim 99 wherein the disulphide-bonded adipocyspin polypeptide is human adipocyspin.

101. A formulation or dosage form of claim 99 wherein the adipocyspin polypeptide is selected from one or more of the following;

i) an adipocyspin polypeptide wherein at least one of the cysteine residues corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues,

ii) an adipocyspin polypeptide as defined in i) wherein two pairs of the cysteine residues are joined in intramolecular disulphide bonds,

iii) an adipocyspin polypeptide wherein three pairs of the cysteine residues are joined in disulphide bonds

iv) an adipocyspin polypeptide as defined in iii) wherein the said adipocyspin polypeptide is human adipocyspin

102. A formulation or dosage form according to claim 99 wherein each of the residues of the adipocyspin polypeptide corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues

103. A formulation or dosage form according to claim 99 wherein each of the residues of the adipocyspin polypeptide corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with those cysteine residues to which they are joined in human adipocyspin

104. A formulation or dosage form capable of delivery of an effective amount of adipocyspin polypeptide when administered or self administered to a human being or other mammal sufficient to decrease the amount of adipose tissue or body mass.

105. A formulation or dosage form according to claim 104 wherein the adipocyspin polypeptide is human adipocyspin.

106. A formulation or dosage form according to claim 104 wherein the adipocyspin polypeptide is selected from one or more of the following;

i) an adipocyspin polypeptide wherein at least one of the cysteine residues corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues,

ii) an adipocyspin polypeptide as defined in i) wherein two pairs of the cysteine residues are joined in intramolecular disulphide bonds,

iii) an adipocyspin polypeptide wherein three pairs of the cysteine residues are joined in disulphide bonds

107. An adipocyspin polypeptide as defined in Claim 106 wherein the said adipocyspin polypeptide is human adipocyspin.

108. A formulation or dosage form capable of delivery of an effective amount of adipocyspin polypeptide when administered or self administered to a human being or other mammal sufficient to decrease the amount of adipose tissue or body mass.

109. A formulation or dosage form according to claim 108 wherein the adipocyspin polypeptide is recombinant, isolated, purified, or synthesized.

110. A formulation or dosage form according to claim 108 wherein the adipocyspin polypeptide is human adipocyspin.

111. A formulation or dosage form according to claim 108 wherein at least one of the cysteine residues of the adipocyspin polypeptide corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues.

112. A formulation or dosage form capable of delivery of an effective amount of adipocyspin polypeptide when administered or self administered to a human being or other mammal sufficient to decrease the amount of adipose tissue or body mass wherein the adipocyspin polypeptide contains disulphide bonds.

113. A method of monitoring the therapy of a mammalian individual predisposed to or suffering from a condition

a. associated with adipocyspin polypeptide regulation;

b. requiring insulin enhancement, or

said method comprising or including the step of monitoring the individual for enhancement of the presence of adipocyspin polypeptide where the adipocyspin polypeptide has one of the following

i) at least one of the cysteine residues corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues,

ii) an adipocyspin polypeptide as defined in i) wherein two pairs of the cysteine residues are joined in intramolecular disulphide bonds,

iii) an adipocyspin polypeptide wherein three pairs of the cysteine residues are joined in disulphide bonds

114. The method according to claim 113 wherein any one or more of the adipocyspin polypeptide isoforms utilized is human adipocyspin.

115. The method according to claim 113 wherein the individual is a human.

116. The method according to claim 113 wherein the condition is hyperglycemia, insulin resistance, metabolic syndromes associated with insulin resistance, Type 2 diabetes mellitus, or obesity, metabolic syndromes including hypertension, artherosclerosis, coronary heart disease, ischemic heart disease, or polycystic ovary syndrome, or a condition associated with increased body mass or adiposity.

117. The method according to claim 113 wherein the adipocyspin polypeptide is obtained from a biological sample.

118. The method according to claim 113 wherein the levels or expression patterns are obtained by quantitating or assessing the expression pattern of adipocyspin.

119. The method according to claim 113 wherein the assessment method utilises electrophoresis, HLPC, or mass spectrometry.

120. The method according to claim 113 wherein the levels or expression patterns are quantitated or assessed using antibodies specific to adipocyspin polypeptide isoforms.

121. The method according to claim 113 wherein adipocyspin polypeptide is obtained by the expression of a recombinant polynucleotide encoding adipocyspin polypeptide in mammalian cells.

122. The method according to claim 113 wherein the recombinant polynucleotide encodes a polypeptide having the sequence as described in Figure ** or a biologically active fragment thereof or a variant thereof.

123. The method according to claim 113 wherein adipocyspin polypeptide is purified from an animal tissue.

124. The method according to claim 113 wherein the animal is a human, mouse, rat, dog, bovine, or a non-human primate.

125. The method according to claim 113 wherein the tissue is serum or adipocytes.

126. The method according to claim 113 wherein the separation comprises a step of electrophoresis.

127. The method according to claim 113 wherein the separation does not comprise a step of electrophoresis.

128. The method according to claim 113 wherein the separation comprises a step of chromatography.

129. The method according to claim 113 wherein the separation does not comprise a step of chromatography.

130. A composition made by a method of preparing a composition comprising adipocyspin polypeptide comprising the steps of;
(a) obtaining a first composition comprising at least two forms of adipocyspin polypeptide that differ in their degree or type of disulphide bonding; and
(b) separating the forms of adipocyspin polypeptide at least to some extent such separation being based on the degree or type of disulphide bonding thereby producing a second composition that differs from the first composition in the adipocyspin polypeptide profile.

131. An antibody specific to the disulphide bonded isoforms of adipocyspin polypeptide selected from the group consisting of
(A) an adipocyspin polypeptide wherein at least one of the cysteine residues corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues,
(B) an adipocyspin polypeptide as defined in (A) wherein two pairs of the cysteine residues are joined in intramolecular disulphide bonds,
and
(C) an adipocyspin polypeptide wherein three pairs of the cysteine residues are joined in disulphide bonds

132. An antibody of claim 131 which is a monoclonal antibody.

133. An antibody of claim 131 which is a capable of two site capture.

134. A composition of matter which comprises an antibody specific to the disulphide bonded isoforms of adipocyspin polypeptide selected from the group consisting of
(A) an adipocyspin polypeptide wherein at least one of the cysteine residues corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of human adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues,
(B) an adipocyspin polypeptide as defined in (A) wherein two pairs of the cysteine residues are joined in intramolecular disulphide bonds,
and
(C) an adipocyspin polypeptide wherein three pairs of the cysteine residues are joined in disulphide bonds

135. A hydridoma specific to the production of antibodies specific to the adipocyspin polypeptide

136. A method of screening an agent or for an agent useful in a mammal for enhancing the level of adipocyspin polypeptide which method comprises administering to the mammal or tissue thereof or one or more cells or to a mammal any enhancement of such adipocyspin polypeptide production by such mammal or mammalian tissue or one or more cells.

137. An agent useful for enhancing the level of adipocyspin polypeptide identified by a method of screening which comprises administering to the mammal or tissue thereof or to a mammal any enhancement of such adipocyspin polypeptide production by such mammal or mammalian tissue.

138. A method of screening for one or more cells capable of expressing a adipocyspin polypeptide comprising identifying and/or determining the level of a specific adipocyspin polypeptide isoform or expression profile of at least two adipocyspin polypeptide isoforms expressed by said cell or cells and identifying and/or purifying and/or isolating said cell or cells.

139. The method according to claim 136 wherein the adipocyspin polypeptide is human adipocyspin.

140. The method according to claim 136 wherein the identification and/or determination of any one or more adipocyspin polypeptides utilises an antibody specific to the disulphide bonding of adipocyspin polypeptide selected from the group consisting of

(A) an adipocyspin polypeptide wherein at least one of the cysteine residues corresponding to cysteine residues 62, 72, 83, 86, 101 and 116 of adipocyspin is joined in an intramolecular disulphide bond with any of the other cysteine residues,
(B) an adipocyspin polypeptide as defined in (A) wherein two pairs of the cysteine residues are joined in intramolecular disulphide bonds,
and
(C) an adipocyspin polypeptide wherein three pairs of the cysteine residues are joined in disulphide bonds

141. The method according to claim 136 wherein the antibody is a monoclonal antibody.

142. Any one or more cells identified and/or isolated and/or purified by a method of screening for one or more cells capable of expressing a disulphide-bonded adipocyspin polypeptide comprising identifying and/or determining the level of a specific adipocyspin polypeptide isoform or expression profile of at least two disulphide-bonded adipocyspin polypeptide isoforms expressed by said cell or cells and identifying and/or purifying and/or isolating said cell or cells.

143. A method of treating a mammalian patient subject to or for obesity or increased adipose tissue mass and/or having any of the characteristics of obesity or increased adipose tissue mass which comprises or includes administering to that patient adipocyspin and/or an agonist thereof.

144. A method of claim 143 wherein the mammal is human and the adipocyspin is human adipocyspin.

145. A method of claim 143 or 144 wherein the adipocyspin is full length.

146. A method of claim 143 wherein said adipocyspin is disulphide-bonded.

147. The method of claim 143 wherein the adipocyspin preparation is formulated in a manner suitable for parenteral administration.

148. A method of treating a mammalian patient to prevent and/or reverse obesity or increased adipose tissue mass and/or any of the characteristics of obesity or increased adipose tissue mass which comprises or includes administering to that patient adipocyspin and/or an agonist thereof.

149. A method of treating a human being subject to obesity or increased adipose tissue mass and/or having any of the characteristics of obesity or increased adipose tissue mass which comprises administering to that patient an effective amount of adipocyspin and/or an agonist thereof.

150. A method of treating a mammalian patient subject to any one or more of hepatic steatosis (fatty infiltration), hepatic inflammation, hepatic necrosis, hepatic fibrosis, hepatic cirrhosis, and/or hepatic dysfunction which comprises or includes administering to that patient adipocyspin and/or an agonist thereof.

151. An article of manufacture comprising or including a vessel containing adipocyspin, and/or an adipocyspin agonist; and instructions for use of adipocyspin, or adipocyspin agonist (for example, as contained within the vessel) for treating, preventing or reversing obesity or increased adipose tissue mass and/or any characteristic of obesity or increased adipose tissue mass.

152. An article of manufacture comprising or including a packaging material containing adipocyspin, and/or an adipocyspin agonist; and instructions for use of adipocyspin, and/or an adipocyspin agonist (for example, as contained within the packaging material) for treating, preventing or reversing obesity or increased adipose tissue mass and/or any characteristic of obesity or increased adipose tissue mass.

153. The use of adipocyspin and/or an adipocyspin agonist in the manufacture with other material or materials (whether recipients, co-actives, diluents or the like and/or whether a dosage unit defining vessel) of a dosage unit or pharmaceutical composition effective for use in the treatment, prevention and/or reversal of obesity or increased adipose tissue mass and/or any characteristic of obesity or increased adipose tissue mass in a mammalian patient (whether human or otherwise).

154. The use of an effective amount of adipocyspin and/or an adipocyspin agonist in the manufacture with other material or materials (whether recipients, co-actives, diluents or the like and/or whether a dosage unit defining vessel) of a dosage unit or pharmaceutical composition effective for use in the treatment, prevention and/or reversal of obesity or increased adipose tissue mass and/or any characteristic of obesity or increased adipose tissue mass in a mammalian patient (whether human or otherwise).

155. A method of treatment of mammalian patient which includes or comprises, in any sequence, the monitoring of the adipocyspin in the mammalian patient and or the administration to the mammalian of adipocyspin and or an agonist thereof, such treatment being for the purpose of treating, reversing, or preventing liver disease obesity or increased adipose tissue mass and or any characteristic of obesity or increased adipose tissue mass.

156. A method of claim 155 wherein the mammalian patient is a human.

157. A method of treatment of mammalian patient which includes or comprises, in any sequence, the monitoring of the adipocyspin mRNA in the mammalian patient and or the administration to the mammalian of adipocyspin and or an agonist thereof, such treatment being for the purpose of treating, reversing, or preventing obesity or increased adipose tissue mass and or any characteristic of obesity or increased adipose tissue mass.

158. A method of claim 157 wherein the mammalian patient is a human.

159. A method of measuring adipocyspin in a mammalian patient which comprises or includes assaying the concentration of adipocyspin in blood or tissue(s).

160. A method of claim 156 wherein the concentration is determined by immunological methods such as radioimmunoassay (RIA), and/or ELISA.

161. A method of claim 156 wherein the tissue is adipose tissue.

162. A method of measuring adipocyspin in a mammalian patient which comprises or includes assaying the concentration of adipocyspin mRNA in blood or tissue(s).

163. A method of claim 162 wherein the concentration of adipocyspin mRNA is determined by RT-PCR, Northern analysis, in situ hybridisation, and/or radioimmunoassay (RIA).

164. A method of claim 163 wherein the tissue is adipose tissue.

165. An assay capable of measuring adipocyspin in a mammalian patient which comprises or includes isolating from the mammalian patient a blood or tissue(s) sample, preparing the sample, assaying the concentration of adipocyspin in blood or tissue(s).

166. A method of claim 165 wherein the concentration is determined by immunological methods such as radioimmunoassay (RIA), and/or ELISA.

167. A method of treating a mammalian patient itself still able to encode for adipocyspin comprising administering to the patient adipocyspin and/or an agonist of the site of action of adipocyspin in a sufficient amount(s) to suppress obesity or increased adipose tissue mass below levels that would have been or likely would have been present without such administration.

168. A method of claim 167 wherein the mammal is a human and the adipocyspin is human adipocyspin.

169. A method of 167 wherein the adipocyspin is full length.

170. A method of treating a mammalian patient itself still able to encode for adipocyspin comprising administering to the patient adipocyspin and/or an agonist of the site of action of adipocyspin in a sufficient amount(s) to suppress obesity or increased adipose tissue mass below levels that would have been or likely would have been present without such administration.

171. A method of claim 170 wherein the mammal is a human and the adipocyspin is human adipocyspin.

172. A method of claim 170 wherein the adipocyspin is full length.

173. The use of adipocyspin and/or an agonist of the site of action of adipocyspin, in the manufacture with other material or material(s) (whether recipients, co-actives, diluents or the like and/or whether a dosage unit defining vessel) of a dosage unit or pharmaceutical composition effective for use to suppress levels of obesity or increased adipose tissue mass below those that would have been or likely would have been present without such administration.

174. The use of adipocyspin and/or an agonist of the site of action of adipocyspin, in the manufacture with other material or material(s) (whether recipients, co-actives, diluents or the like and/or whether a dosage unit defining vessel) of a dosage unit or pharmaceutical composition effective for use in the treatment, prevention and/or reversal of levels of obesity or increased

adipose tissue mass or disorder and/or having the characteristics of obesity or increased adipose tissue mass.

175. An article of manufacture comprising or including a vessel containing adipocyspin and/or an agonist of the site of action of adipocyspin; instructions for use of adipocyspin and/or an agonist of the site of action of adipocyspin, (for example, as contained within the vessel) effective for use to suppress levels of obesity or increased adipose tissue mass levels below those that would have been or likely would have been present without such administration.

176. An article of manufacture comprising or including a vessel containing adipocyspin and/or an agonist of the site of action of adipocyspin; instructions for use of adipocyspin and/or an agonist of the site of action of adipocyspin (for example, as contained within the vessel) for treating, preventing or reversing levels of obesity or increased adipose tissue mass and/or any of the characteristics of obesity or increased adipose tissue mass.

DATED THIS 28 DAY OF October 2003
AJ PARK
PER C Palmer
AGENTS FOR THE APPLICANT

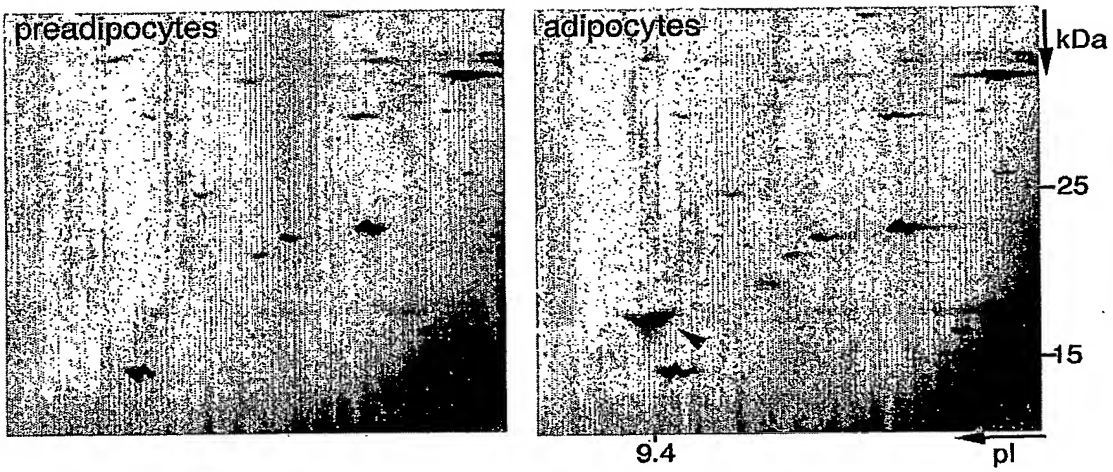
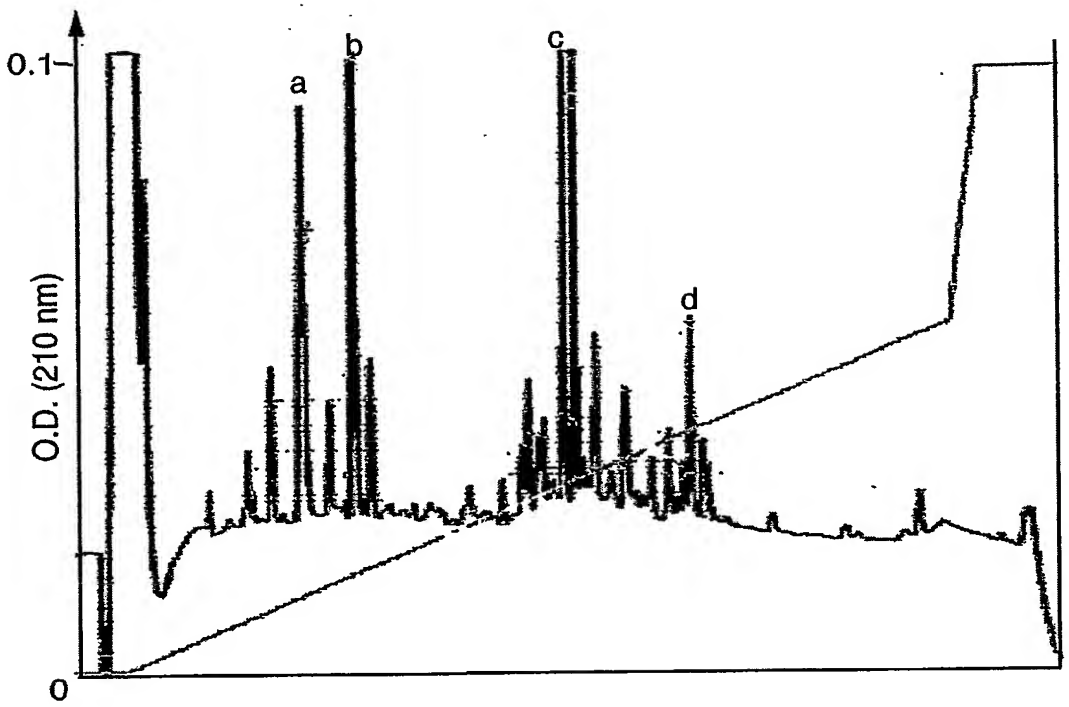


Figure 1



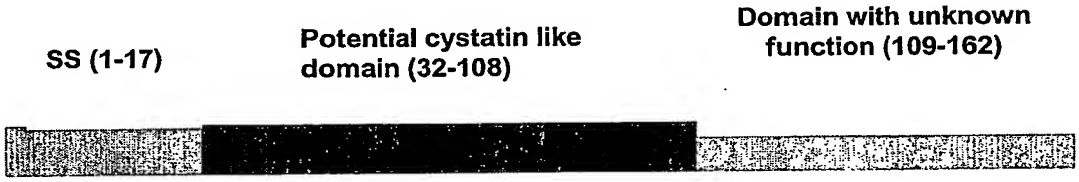
Farctions	Amino acid sequences
a	LQQTNCPK
b	GTEPELSETQR
c	CLLISLALWLGTVGTR
d	HPPVQLAFQEIGVDR

Figure 2

A. SEQENCE ID NO:1

MKCLLISLALWLGTVGTRGTEPELSETQRRSLQVALEEFHKHPPVQLA
FQEIGVDRAEEVLFSAGTFVRLEFKLQQTNCPKKDWKKPECTIKPNGR
RRKCLACIKMDPKGKILGRIVHCPILKQGPQDPQELQCIKIAQAGEDP
HGYFLPGQFAFSRALRTK

B.



C.

Adipocyspin

LQVALEEFHKHPPVQLAFQEIGVDRAEEVLFSAGTFVRLEFKLQQTNCPKKDWKKPE-CT

90

Cystatin C

ADFAVAEYNKKSNDLYELRVIKVVSAKSQVV-AGTNYYIKVEVGETNGKKLSPTDLENCP 78

Query: 91

IKPNCRRRKCLACIKMDP 108

Sbjct: 79

FLEQCEEGFCDFEVWEKP 96

Figure 3

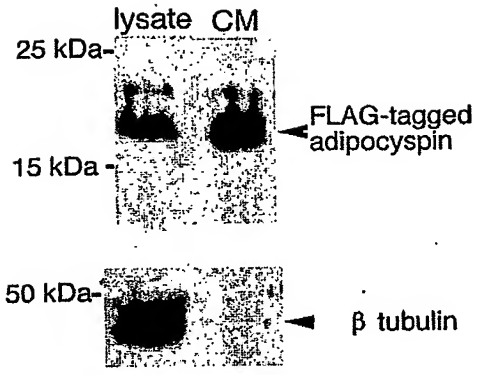


Figure 4.

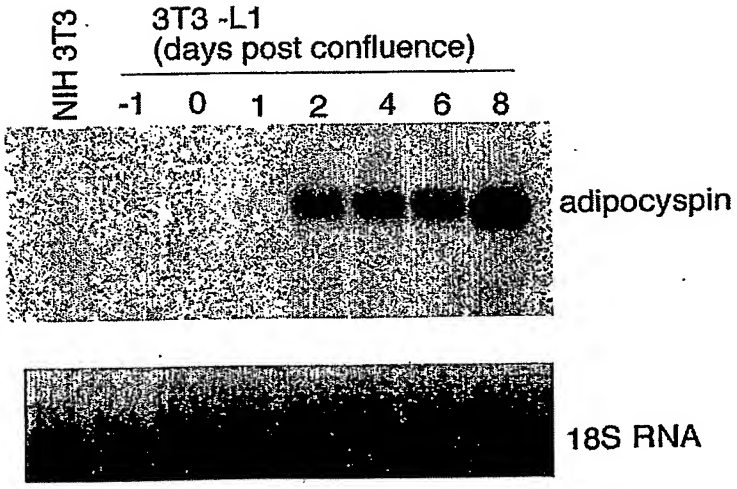


Figure 5.

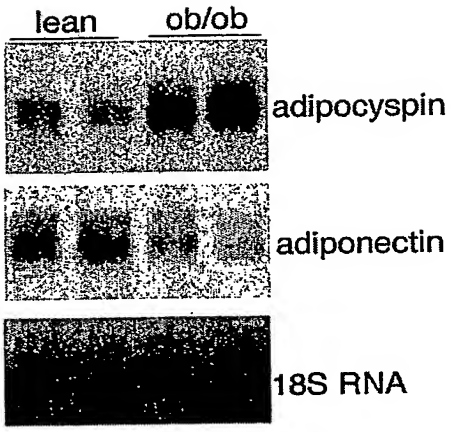


Figure 6

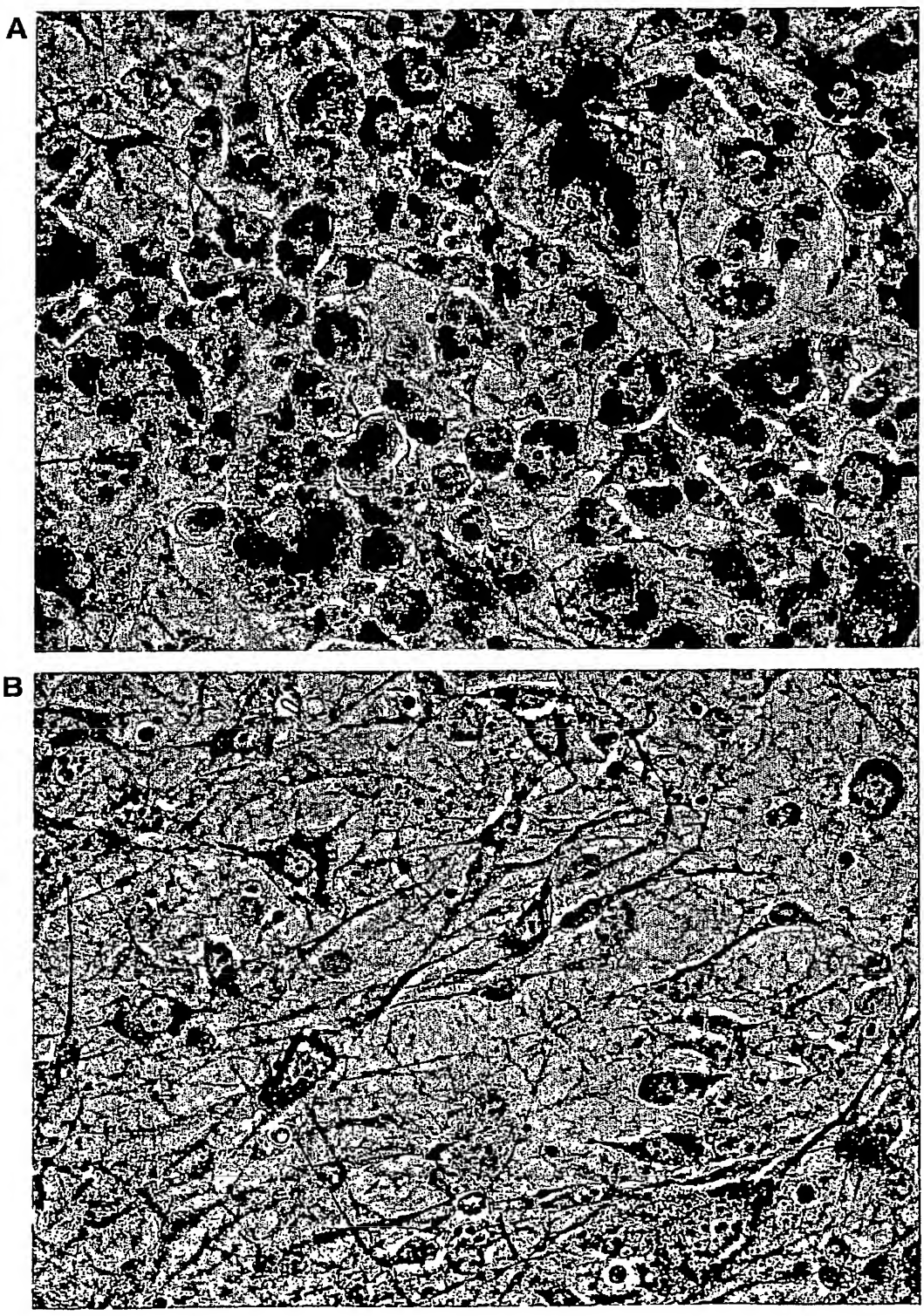


Figure 7.

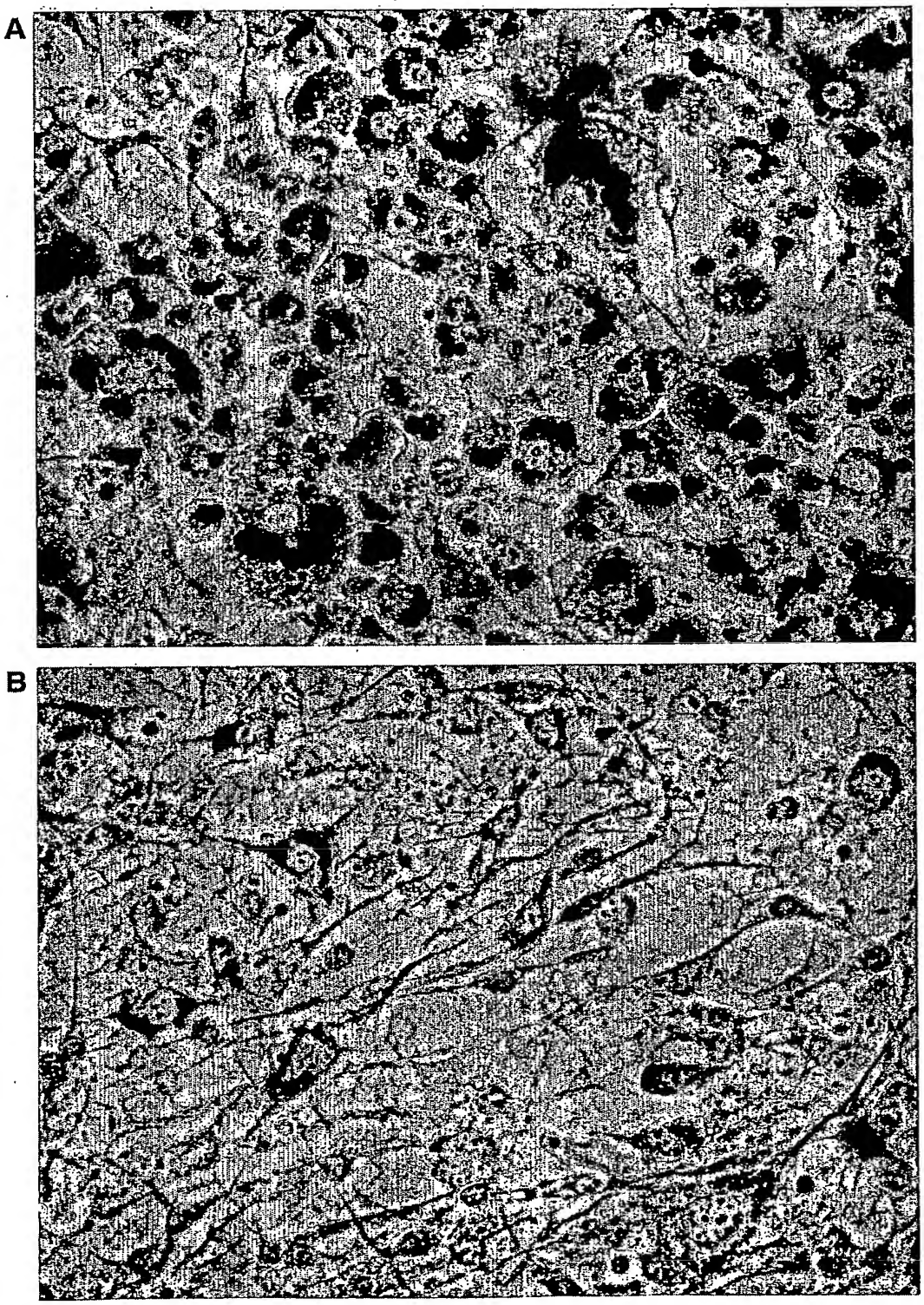


Figure 7.

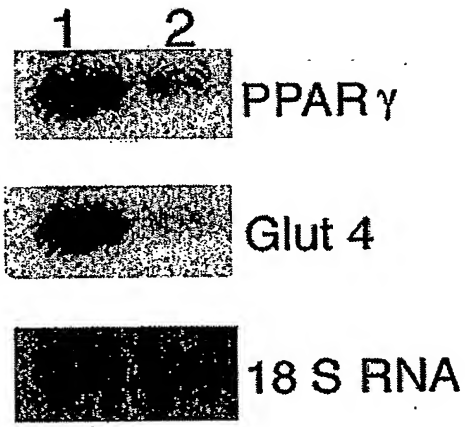


Figure 8.

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